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Poster Abstracts

Adaptive Immunity

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Dopamine receptor D5 expressed on dendritic cells promotes $CD4^+$ T-cell-mediated autoimmunity

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Dendritic cells (DCs) are responsible for priming T-cells and for promoting their differentiation from naïve T-cells into appropriate effector cells . Emerging evidence points toward neurotransmitters not only mediates interactions into the nervous system, but can also contribute to the modulation of immunity. Accordingly, we have analyzed the role of dopamine in the regulation of DCs function.

Our results show that DCs express the machinery necessary to synthesize and to store dopamine. They also express dopamine receptors D1 (D1R), D2R, D3R and D5R, but only D5R is significantly down-regulated after LPS-induced maturation. *In vitro* experiments indicate that lack of D5R in DCs impairs LPS-induced IL-12 secretion and consequently attenuates activation and proliferation of antigen-specific $CD4^+$ T-cells in co-culture experiments. To determine the relevance of D5R expressed on DCs *in vivo*, we studied the role of this receptor in the modulation of a $CD4^+$ T-cell-driven autoimmunity. Importantly, D5R-deficient DCs prophylactically transferred to a WT recipient were able to reduce the severity of Experimental Autoimmune Encephalomyelitis (EAE). Furthermore, we examined the phenotype of the $CD4^+$ T-cells infiltrated into the central nervous system during the peak of the disease. Mice transferred with D5R-deficient DCs showed a significant reduction in the percentage of infiltrating Th17 cells without differences in the percentage of Th1 cells when compared to animals transferred with wild-type DCs.

Our findings demonstrate that D5R expressed on DCs, by contributing to $CD4^+$ T-cell activation and differentiation to Th17 phenotype, is able to modulate the development of an autoimmune response *in vivo*.

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Deletion of the signal transduction molecule p14 under the CD11c promotor impairs the development of the murine Langerhans cell network

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Aim: Dendritic cells (DC) are important regulators of immunity and tolerance. To fulfil their antigen presenting capacity, DC need to process and distribute incorporated antigen via endosomal sorting to distinct cellular compartments so that they can present it to effector T cells. The extracellular signaling-regulated kinase (ERK) cascade is involved in endosomal sorting processes. Hence, we investigated the role of the adaptor molecule p14, an essential part of the ERK cascade, in the context of DC function.
Methods: We generated a DC specific knock out mouse model by Cre-CD11c-mediated ablation of p14. Phenotypical analysis of the DC populations was carried out by flow cytometry analysis as well as with immunofluorescence microscopy of epidermal sheets and cryostat sections.
Results: We noted greatly diminished numbers within the fraction of migrating DC in the skin-draining lymph nodes of both Langerhans cells and $langerin^+$ $CD103^+$ dermal DC. The reduced number of skin DC, especially epidermal Langerhans cells was further confirmed by quantitative and qualitative analysis of the skin. Investigating the ontogeny of Langerhans cells by analysing the skin of newborn mice, revealed, that Langerhans cells are capable of populating the epidermis within 3 days after birth. However, the maintainance and homeostasis of the network is affected in p14 knock-out mice as indicated by a constant loss of Langerhans cells starting within 1 week after birth. The responsible mechanisms are being studied.
Conclusion: In summary, our observations identify p14 as an important molecule regulating the homeostasis of the Langerhans cell network.

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Dendritic cell: T cell interaction in the liver during Th2-mediated fibrotic disease

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Dendritic cells (DCs) are professional APCs that recognise and respond to pathogens and orchestrate T cell polarisation. While DC:T cell interaction is well characterised for Th1-inducing pathogens, it is much less defined in the context of Th2-inducing pathogens. We have addressed the role of DCs during murine infection with the medically important parasitic helminth *Schistosoma mansoni,* which causes chronic disease that is associated with a potent Th2 response and severe liver fibrosis. We have characterised the interaction of DCs and T cells within the liver, a major site of *S. mansoni* egg deposition and granuloma formation leading to fibrosis and disease pathology. Using flow cytometry and thin section confocal microscopy, we demonstrate that DCs and T cells accumulate in the liver prior to egg deposition and colocalise within and around *S. mansoni* granulomas by 6 weeks post-infection, with development of granulomatas and severe fibrosis evident from week 8. Depletion of $CD11c^+$ DCs resulted in a loss of T cell homing to the infected liver coincident with impaired Th2 development. Ongoing work is assessing the impact of this altered granuloma formation on fibrosis, alternative activation of liver macrophages, and the organisation of T and B cells within the secondary lymphatics.

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Structural and biophysical binding parameters that govern T-cell antigen discrimination

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T-cells direct adaptive immune responses through a specific interaction between the clonotypically expressed T-cell receptor (TCR) and peptide-major histocompatibility complex (pMHC) antigens on the target cell surface. The molecular rules that drive MHC restriction, and allow T-cells to differentiate between peptides derived from healthy and diseased cells, remain poorly understood. Thus, there exist a number of different, and sometimes conflicting, models that describe the structural and biophysical determinants that govern T-cell reactivity.

We have recently determined the atomic structures and biophysical properties of a range of anti-viral, anti-tumour and anti-self specific TCRs in complex with their cognate antigen, and a range of antigenic variants. Using T-cell clones that express these specific TCRs, we have investigated the molecular rules that govern antigen recognition in these systems. Our new data reveal important insights into how the antigenic origin of peptide epitopes affects TCR/pMHC binding parameters and the quality of T-cell responses. Furthermore, these studies have furthered our understanding the principles that govern pMHC recognition by T-cells. Overall, our findings will contribute towards improved strategies for the rational development of immunotherapeutic approaches, such as vaccines, for the treatment of infectious diseases, cancer and autoimmune disease.

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CTLA-4 activates the hippo pathway to regulate terminal differentiation of the CD8$^+$ T cell

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Replicating, antigen-specific CD8$^+$ T cells must not commit to terminal differentiation until there has been sufficient clonal expansion. The Hippo pathway of organ size control mediates this requirement by linking expression of the differentiation-inducing transcription factor, Blimp-1, to contact between replicating cells, which would be dependent on their frequency. TCR and IL-2R stimulation assemble the Hippo pathway in the CD8$^+$ T cell by inducing expression of WW45, Mob1, Lats1, and YAP, the transcriptional co-activator that mediates organ growth. Contact between activated CD8$^+$ T cells triggers the Hippo pathway, causing serine phosphorylation and degradation of YAP. This is suppressed by addition of naïve CD8$^+$ T cells, indicating that the ligand-receptor pair triggering the Hippo pathway is expressed only by activated cells. The ligand was identified by suppressing YAP degradation with blocking CD80/86 antibody, and the receptor was defined by inducing YAP degradation by crosslinking CTLA-4. That YAP regulates differentiation was shown by ectopically expressing a non-phosphorylatable, stable form of YAP in activated CD8$^+$ T cells, which suppressed Blimp-1 expression *in vitro*, and the differentiation/senescence marker, KLRG1, *in vivo*. This role for CTLA-4 was confirmed by the presence of YAP in T cells from CTLA-4−/−, but not CTLA-4+/−, mice and similar suppression of KLRG1 *in vivo*. Therefore, in a process that resembles quorum sensing, the Hippo pathway regulates terminal differentiation of the CD8$^+$ T cell.

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CD3 limits the efficacy of TCR gene therapy *in vivo*

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The function of T cell receptor (TCR) gene modified T cells is dependent on efficient surface expression of the introduced TCR a/b heterodimer. We tested whether endogenous CD3 chains are rate-limiting for TCR expression and antigen-specific T cell function. We show that co-transfer of CD3 and TCR genes into primary murine T cells enhanced TCR expression and antigen-specific T cell function *in vitro*. Peptide titration experiments showed that T cells expressing introduced CD3 and TCR genes recognised lower concentration of antigen than T cells expressing TCR only. *In vivo* imaging revealed that TCR + CD3 gene modified T cells infiltrated tumors faster and in larger numbers, which resulted in more rapid tumor elimination compared to T cells modified by TCR only. Following tumor clearance, TCR+CD3 engineered T cells persisted in larger numbers than TCR-only T cells and mounted a more effective memory response when re-challenged with antigen. The data demonstrate that provision of additional CD3 molecules is an effective strategy to enhance the avidity, anti-tumor activity and functional memory formation of TCR gene modified T cells *in vivo*.

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Th1/Th17 cell induction following vaccination with the novel tuberculosis vaccine MVA85A and their role in *Mycobacterium tuberculosis* challenge

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The efficacy of Bacille Calmette-Guérin (BCG), the current vaccine against tuberculosis (TB), is variable, ranging from 0% to 80%. Interferon (IFN)-γ has long been known to be essential for protection against TB disease, however data is now emerging on a role for interleukin (IL)-17 in both murine and bovine TB vaccine studies, as well as in humans.

MVA85A is a novel investigational vaccine designed to enhance BCG. Here we show that MVA85A induces a population of IFN-γ^+IL-17$^+$ CD4$^+$ T cells in mice, and that mucosal vaccination induces higher levels than systemic vaccination. Co-administration of cholera toxin, an adjuvant known to induce IL-17 production, with BCG, and subsequent boosting with MVA85A enhances secretion of IL-17 from both single-positive and IFN-γ^+IL-17$^+$ CD4$^+$ T cells. After aerosol *Mycobacterium tuberculosis* challenge, BCG+ cholera toxin prime - MVA85A boost vaccinated animals have lower CFU counts than BCG - MVA85A vaccinated animals. This suggests that IL-17 may play a role in MVA85A-induced protection against TB disease.

These studies are important to developing an understanding the mechanisms of protection against TB disease and aim to uncover immune responses that could be targeted in the quest for an effective TB vaccine.

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The role of cytokine and TCR signaling in the development of regulatory T cells

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FoxP3$^+$ regulatory T-cells (Treg) are essential for preventing autoimmunity by the immune system. The dynamics and signalling requirements for Treg development in the thymus are not well understood. To investigate this, we used mice bearing a FoxP3 reporter allele (GFP^{FoxP3}), in which Zap70 expression is controlled by a Tet-inducible transgene (TetZap70) that is induced by administration of antibiotic doxycycine (dox). Zap70 deficient thymocytes are arrested at the CD4$^+$ CD8$^+$ double positive stage of development. Induction of Zap70 expression by dox therefore restores positive selection. In timecourses of Zap70 induction of TetZap70 GFP^{FoxP3} mice, we found that Treg develop after day 3. T_{reg} remained in the thymus until d7, at which time GFP$^+$ Treg were first detected in peripheral lymphoid organs.

Previous studies have described a role for the cytokines IL-2 and TGFβ for the intrathymic induction of FoxP3. Using the TetZap70 GFP^{FoxP3} mice we investigated the temporal requirement for these cytokines during T_{reg} development. Through the use of blocking antibodies and the addition of cytokine-antibody complexes we observed T_{reg} emerging CD25^{Low}. T_{reg} then increased CD25 expression levels in an IL-2 dependant manner, contrary to previous models. Additionally, despite a recognised role in generation of Treg from mature T cells, we found no role for TGFβ in thymic Treg development.

We therefore propose an alternative model for the thymic induction of FoxP3 in which TCR signals alone are sufficient to induce FoxP3 expression but that continued development of Treg is reinforced by IL-2.

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The PI3Ks p110α and p110δ control regulatory T cell function

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CD4$^+$ Foxp3$^+$ regulatory T cells (Tregs) play a crucial role in the maintenance of self-tolerance and suppression of potentially harmful immune responses. It has previously been shown that mice expressing functionally inactive p110δ, a class Ia PI3-kinase isoform, have reduced proportions of Tregs with impaired suppressive function. However, a parallel abnormality in Th cells in these mice precludes a definitive interrogation of the role of p110δ in Tregs *in vivo*. Moreover, p110α has also been implicated in regulatory function, but the interplay between the isoforms remains unclear. Our work aims to resolve these issues, using mice that express Cre-recombinase downstream of Foxp3 to delete floxed p110 isoforms specifically in Tregs.

Here we show that mice with Tregs lacking p110δ, or both p110δ and p110α, have histopathology indicative of Treg deficits, including increased lymphoid hyperplasia and intestinal inflammatory cell infiltration. Treg proportions are reduced in secondary lymphoid organs. Tregs from both mice express lower levels of CD38, a marker of highly suppressive Tregs. Accordingly, the prevalence of 'antigen-experienced' CD4$^+$ T cells and dendritic cell activation are increased. These data suggest an intrinsic role for p110 isoforms in Treg function, and results from preliminary immunisation studies and *in vitro* suppression assays support this assertion. Furthermore, it appears deletion of p110α in Tregs may augment the effects of p110δ deletion alone. We are currently elaborating upon these observations by using experimental autoimmune encephalitis to investigate the Treg-intrinsic roles of p110α and p110δ in autoimmunity, and preliminary data supports our *in vitro* findings.

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Evasion of immunity to *Plasmodium falciparum* malaria by IgM masking of protective IgG epitopes in PfEMP1

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Plasmodium falciparum malaria is a major cause of mortality and severe morbidity. Its virulence is related to the parasite's ability to evade host immunity through clonal antigenic variation and tissue specific adhesion of infected erythrocytes (IEs). The *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family is central to both. Here, we present evidence of a *P. falciparum* evasion mechanism not previously documented: the masking of PfEMP1-specific IgG epitopes by nonspecific IgM. Nonspecific IgM binding to erythrocytes infected by parasites expressing the PfEMP1 protein VAR2CSA (involved in placental malaria pathogenesis and protective immunity) blocked subsequent specific binding of human monoclonal IgG to the DBL3X and DBL5ε domains of this PfEMP1 variant. Strikingly, a VAR2CSA-specific monoclonal antibody that binds outside these domains and can inhibit IE adhesion to the specific VAR2CSA receptor chondroitin sulfate A was unaffected. Nonspecific IgM binding protected the parasites from FcγR-dependent phagocytosisof VAR2CSA+ IEs, but it did not affect IE adhesion to chondroitin sulfate A or lead to C1q deposition on IEs. Taken together, our results indicate that the VAR2CSA affinity for nonspecific IgM has evolved to allow placenta-sequestering *P. falciparum* to evade acquired protective immunity without compromising VAR2CSA function or increasing IE susceptibility to complement-mediated lysis. Furthermore, functionally important PfEMP1 epitopes not prone to IgM masking are likely to be particularly important targets of acquired protective immunity to *P. falciparum* malaria.

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Comparison of the safety and immunogenicity of a candidate TB vaccine, MVA85A, given by the intramuscular or intradermal route in BCG-vaccinated adults

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MVA85A, a recombinant Modified Vaccinia virus Ankara expressing the *Mycobacterium tuberculosis* antigen 85A, can improve BCG-induced protection in pre-clinical animal models and is highly immunogenic in BCG-vaccinated volunteers. MVA85A has been administered intradermally (ID) in all human trials, with no vaccine-related serious adverse events, however, if it proves to be safe and immunogenic by the intramuscular (IM) route, then this may be the preferred route in subsequent trials.

In this Phase 1 clinical trial, 24 healthy, BCG-vaccinated adults received 1×10^8 pfu of MVA85A, either by IM or ID injection. The primary immunological readout was the ex-*vivo* IFN-γ ELISpot, performed on fresh PBMC at screening and 7, 14, 28, 84 and 168 days post-vaccination, stimulating with overlapping pools of antigen 85A peptides. Safety of the vaccine was assessed at all time points.

MVA85A, given by the IM route, was safe and well tolerated in 12 healthy adults and produced a potent immune response to antigen 85A peptides, not significantly different to that induced by the ID route. Gene expression studies also found there to be no significantly differentially expressed genes between the two routes. Further flow cytometry is planned to fully characterise the immune response induced by these routes.

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Expression of lineage defining transcription factors in dual cytokine secreting human $CD4^+$ T cells

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$CD4^+$ T helper (Th) subsets can be defined by the cytokines they produce and their transcription factor expression. The paradigm of Tbet and IFNγ denoting Th_1 cells, GATA-3 and IL-5, IL-4 and IL-13 indicating a Th_2 phenotype, and RORC and IL-17 signifying a Th_{17} cell is well established. Initially these subsets were thought to have a stable phenotype however there are small subsets of cells that co-express cytokines normally attributed to separate lineages (e.g. IFNγ^+IL-17^+, IFNγ^+IL-5^+ etc.). The expression of the lineage defining transcription factors in these dual cytokine secreting subsets, especially in humans, has not been fully investigated. At a single cell level using multi-colour flow cytometry we wished to determine if the expression of two lineage-defining cytokines is mirrored by expression of both lineage-defining transcription factors.

We have shown that Tbet was expressed in cells secreting IFNγ in combination with other cytokines (IL-10, IL-5 and IL-17) at similar levels to those cells producing IFNγ only. This was also the case for cells expressing IL-5 and GATA-3. Consequently there was co-expression of the lineage defining transcription factors Tbet and GATA-3 in cells that co-express IL-5 and IFNγ. The results for IL-17 and RORC however were inconclusive. Interestingly there was a significantly lower level of IFNγ being produced per cell when co-expressed with either IL-17 or IL-5 compared to cells secreting IFNγ only. We are currently investigating if these changes in IFNγ expression in dual cytokine-secreting cells may be due to changes in transcription of the IFNγ gene.

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High throughput VDJ repertoire analysis to analyse the relationship between subsets of memory B cells

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B cell repertoire diversity is vital for a healthy immune system and is achieved by rearrangement of IGHV, IGHD and IGHJ regions to encode an antibody heavy chain, which is then used in combination with a kappa or lambda light chain comprising IGKV and IGKJ or IGLV and IGLJ genes. A number of factors can change the repertoire of a population; positive selection results in expansion of cells that recognise a particular challenge, and negative selection is a key feature of self tolerance whereby B cells carrying an autoreactive BCR are deleted or undergo receptor editing. We have used high throughput sequencing analysis of the repertoire from phenotypically different populations of B cells to determine their relationship to each other. Our results cast doubt on the validity of CD27 as a marker of memory B cells, and we have also shown that the repertoires of different B cell populations can change with age.

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NK cell regulates T cell priming during influenza A/H1N1 infection

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An effective immune response against influenza A infection depends on generation of virus-specific adaptive immunity. Natural Killer (NK) cells are one of the first-line defenses against influenza. We set out to delineate the role of NK cell in T cell immunity in influenza infection. With a mouse model using Influenza A/PR/8/34, we show that T cell activation mainly occurs in the posterior MLN (pMLN). Depletion of NK cells significantly impairs both DC and T cell recruitment into the posterior MLN. Similar reduction of T cell recruitment is observed when DC migration is blocked by pertussis toxin, suggesting that NK cells could mediate T cell recruitment indirectly through their effects on DC migration. This appears that T cell recruitment depends on IFN-g and transfer of IFN-g competent lymphocyte activated killer cells into IFN-$g^{-/-}$ mice can restore T cell recruitment. Additionally, NK depletion reduces the uptake and presentation of viral antigen by DCs and significantly impaired virus-specific T cell responses. Both IFN-$g^{-/-}$ and perforin$^{-/-}$ mice show reduced influenza antigen uptake by DCs, suggesting that the ability of NK cells to influence DC antigen presentation is mediated by both IFN-g and perforin-mediated mechanisms. These observations suggest that NK cells play a critical role in the uptake and transport of influenza virus and its capacity to prime the adaptive immune response against influenza.

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Efficient generation of anti-viral CD8$^+$ T cell immunity is dependent on the death receptor 3/TL1A pathway

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Aims: Death receptor 3 (DR3, TNFRSF25), the closest family relative to tumour necrosis factor receptor 1 (TNFR1), promotes a number of CD4$^+$ T cell-driven inflammatory diseases. We investigated whether the DR3/TL1A pathway plays a role in CD8$^+$ T homeostasis and anti-viral immunity.
Methods: We used the murine cytomegalovirus (MCMV) and recombinant vaccinia virus (rVV) models of infection in DR3-wildtype and DR3-deficient mice. The expression and role of DR3 on purified murine T-cells stimulated *ex-vivo* was also studied.
Results: We show that DR3 is expressed by naïve CD8$^+$ T cells, with TCR activation increasing its cell surface levels and altering the ratio of DR3 mRNA splice variants. Both CD4$^+$ and CD8$^+$ T cell responses were dramatically reduced in DR3-deficient mice during acute infection. DR3-dependent expansion of virus specific CD8$^+$ T cells in response to MCMV challenge was driven by enhanced proliferation, not decreased cell death, and appeared CD4-independent. Importantly, impaired cellular immunity in virus-infected DR3-deficient hosts was associated with elevated MCMV viral loads during the acute phase of MCMV infection.
Conclusion: This is the first description of DR3 regulating pathogen-specific T cell function *in vivo* and implicates the DR3/TL1A pathway as a potential target for boosting anti-viral T cell immunity.

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Rapid, column-free two-step procedure for the enrichment of human Th17 cells from peripheral blood

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Human Th17 cells are key drivers of autoimmune and allergic reactions. They can be characterized by expression of surface receptors including CCR6, CCR4, CD161 and IL-23R, but lack of CXCR3 expression. They produce IL17 cytokines, are typically IL-17$^+$IFN-γ^- and express the lineage-specific transcription factor RORC2.

A major disadvantage of current isolation methods is the requirement for previous *in vitro* stimulation. We have developed a two-step EasySep^{TM} immunomagnetic column-free method for the enrichment of CD4+ CXCR3− CCR6$^+$ cells from fresh peripheral blood nucleated cells. Firstly, non-CD4 T-cells and CXCR3+ cells are targeted for depletion using dextran-coated magnetic particles and a cocktail of antibody complexes. Labeled cells are separated using an EasySep^{TM} magnet, and pre-enriched CD4 T-cells are poured off. Next, CCR6+ cells are positively selected from the pre-enriched fraction. Labeled cells remain in the tube in the magnet while unwanted cells are then poured off. The procedure can be fully automated using RoboSep^{TM}. Starting with a frequency of 5 ± 2% CD4+CXCR3−CR6+ cells, purities of 94 ± 3% (n = 10) are obtained. Enriched cells show increased levels of IL17 secretion (minimal IFN-γ) by ELISA and intracellular staining and increased RORC2 mRNA expression over total CD4 or CD4$^+$CXCR3$^+$ T-cells. Enrichment of unstimulated human Th17 cells enables the investigation of adaptive immune responses and regulation mechanisms required for the development of future therapies.

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A simple and rapid method for the isolation of untouched human naive and memory CD8 T cells

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CD8 T cells play a central role in the adaptive immune response to intracellular pathogens. The human CD8 T cell pool is comprised of several phenotypically and functionally distinct sub-populations, including naïve cells that have not encountered antigen and express CD45RA and CCR7, and previously activated memory cells, primarily defined by CD45RO expression. Current protocols for the isolation of these populations are time-consuming and require the use of columns. We have developed two new kits for easy and rapid isolation of naïve (CD45RA+CCR7+) and memory (CD45RO+) CD8 T cells from PBMCs by immunomagnetic, column-free cell separation (EasySep^{TM}). Non-CD8 T cells and unwanted CD8 T cell subsets are targeted for depletion by antibody complexes crosslinked to dextran-coated magnetic particles. The labeled cells are separated using an EasySep^{TM} magnet and the desired fraction is poured off. Each procedure is performed in less than an hour and can be fully automated using RoboSep^{TM}. The mean enrichment purities for the naïve and memory CD8 T cell kits are 89 ± 3% and 86 ± 4, respectively. The kits provide a simple and efficient means of isolating untouched human naïve and memory CD8 T cells that are ideal for downstream assays.

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Human Th17 responses: the effect of T-cell density

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Both the cytokine milieu and quality of T-cell activation are capable of determining effector CD4$^+$ T-cell development. We recently found that low strength T-cell activation promotes Th17 responses via a Ca^{2+}/NFATc1 dependent mechanism. Here we extended these investigations by assessing the effect of T-cell density on Th17 responses. Memory CD4$^+$ T-cells were activated with anti-CD3/anti-CD28 beads at a high strength stimulus of 1:1 cell:bead ratio, in the presence of proTh17 cytokines IL-1β, TGF-β, and IL-23. Cell density was decreased from 1×10^6 cells/ml to 0.0625×10^6 cells/ml, maintaining a 1:1 cell:bead ratio. After 6 days the number of IL-17- and IFN-γ-producers were determined by intracellular cytokine staining and flow cytometry. Our results demonstrate that low cell density significantly increased both the proportion and absolute numbers of IL-17$^+$ cells. Titration of proTh17 cytokines revealed that the effect was not due to excess cytokine availability. Furthermore, Th1 responses were unaffected by cell density, suggesting an IL-17-selective effect of cell density. STAT3 and Aryl hydrocarbon Receptor (AhR) are two transcription factors involved in promoting Th17 responses. Previous studies have indicated that STAT3 and AhR activation and expression may in part be dictated by cell density. We found an increased level of STAT3 phosphorylation and AhR expression in low density T-cells, compared to high density T-cells, possibly explaining how activation of T-cells at low density favours Th17 responses. These findings provide new insights into the complex range of factors capable of affecting Th17 responses, and have important implications for *in vitro* differentiation models.

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Foal immunodeficiency syndrome (FIS) – Identifying the genetic basis

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FIS is characterised by weakness, anaemia and severe multiple opportunistic infections of young foals. FIS is 100% fatal within 6–12 weeks; no treatments are effective. The profound anaemia is non-regenerative and PCVs may be as low as 3%. Circulating PMN and T lymphocytes are normal in number and function. However, there is an almost complete lack of circulating B-lymphocytes and circulating immunoglobulins, and reduced numbers of B lymphocytes in spleen and lymph nodes.

This novel immunodeficiency is an autosomal recessive genetic disease and carriers (heterozygotes) are clinically normal; homozygotes all die within a few weeks of birth. Hence, it was important to identify the FIS genetic lesion(s), develop a DNA-based diagnostic test and screen equine populations.

A panel of 286 equine microsatellites identified a large suspect region on chromosome 26 as the most likely candidate. A Genome Wide Association Study (GWAS) using the Illumina 54K Equine SNP chip confirmed and narrowed the critical region to 1.2 Mb. This region was captured by a NimbleGen Sequence Capture array and sequenced (Roche 454). From this, one SNP survived critical analysis being homozygous in FIS foals, heterozygous in obligate FIS carriers and absent in normal ponies. This SNP is exonic and results in an amino acid change in SLC5A3. A diagnostic test for the SNP is now available.

Population studies indicate 42–48% of Fell ponies, 10–18% of Dales ponies and 2% of Coloured ponies are carriers. It is now feasible to develop breeding regimes to eradicate FIS from equine populations.

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The role of pTα isoforms in αβ T cell development

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αβ T cells are heterogenous with respect to their thymic selection and function. Commitment to the αβ lineage requires progression through the β-selection checkpoint, a transition that requires surface expression of the preTCR by immature thymocytes. The preTCR is composed of a rearranged TCRβ chain, the invariant pTα chain, and various CD3 signalling molecules. The availability of two alternatively-spliced pTα isoforms (referred as $pT\alpha^a$ and $pT\alpha^b$), provide the cell with the choice between two distinct preTCR complexes ($preTCR^a$ and $preTCR^b$). However, the truncated form; $pT\alpha^b$, has been frequently omitted in studies or described as non-functional despite previous work suggesting the opposite. Here, we have hypothesized that the heterogeneity of αβ T cell subsets is mainly driven by the presence of the two isoforms of pTa at the β-selection checkpoint. We have begun to investigate this by generating transgenic mice in which a BAC $pT\alpha^a$ transgene was re-introduced into a pTα-deficient background that lacks both pTα isoforms. Together, the preliminary results presented here suggest that $pT\alpha^a$ alone is sufficient for the development of conventional ab T cell subsets such as $CD4^+$ and $CD8\alpha\beta^+$ T cells, and T regulatory cells. However, pTa^a does not appear to rescue development of unconventional $CD8\alpha\alpha^+$ $TCR\alpha\beta^+$ IELs of the small intestine. The implications for development of different populations of αβ T cells will be discussed.

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How old are *your* MHC proteins? The role of protein turnover in MHC codominance

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Allelic variants of MHC glycoproteins are codominantly expressed, but how this is achieved biochemically remains unclear. MHC polymorphism affects protein maturation, but whether the destruction rates of MHC proteins are also allele-dependent has been explored less. To address this, dendritic cells (DCs) or B cell lines were labelled with heavy water (^2H_2O). MHC molecules were immunoprecipitated, and peptide mass isotopomer distributions of tryptic peptides quantified by OrbiTrap MS. The distributions, which shift during ^2H_2O labelling, were used to calculate fractional protein synthesis and turnover rates determined after correcting for cell growth and protein expression changes. EBV-B cells exhibited slow MHC class I and II turnover; faster turnover was observed for maturation intermediates and in peptide loading mutants. Immature monocyte-derived DCs, myeloid and mouse B lymphoma cell lines exhibited substantial MHC protein turnover. Known differences in turnover between HLA class I isotypes (HLA-C > B ≃ A) and LPS-mediated shutdown of MHC class II turnover in DCs were confirmed. Allelic polymorphism had little or no effect on MHC protein turnover, as judged by analysis of allele-specific peptides. In conclusion, the ^2H_2O labelling/Orbitrap peptide MS approach for measuring protein turnover has been validated. MHC protein turnover rates depend on the MHC class and isotype and on cellular context, but are surprisingly allele-independent, suggesting mechanistic or functional constraints. The molecular regulation and functional consequences of MHC protein turnover remain to be elucidated. Exceptional cases (HLA-B27, $H2\text{-}A^{g7}$) merit further study in the context of autoimmune pathogenesis. *In vivo* studies in mice are underway.

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Interleukin 12 mediated recovery of effector cytokine production in hepatitis B virus-specific CD8+ T cells

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CD8 T lymphocytes are vital in resolving HBV infection. However, in chronically infected patients virus-specific CD8 T cells are depleted by Bim-mediated attrition, and remaining cells are functionally exhausted. Previously we found that in many patients HBV-specific T cell responses can be enhanced by blocking the highly expressed co-inhibitory marker cytotoxic T lymphocyte antigen 4 (CTLA-4), which interferes with stimulatory signalling via CD28. However, in other patients, blocking CTLA-4 alone or even in combination with PD-1 was not sufficient to recover responses.

We therefore investigated the effect of using third signal cytokines to maximise T cell activation. Our results show that IL-12 but not IFNα significantly increases IFNγ and TNFα production by HBV-specific CD8 T cells. At the same time IL-12 down-regulates the co-inhibitory molecule PD-1 and the proapoptotic molecule Bim.

IL-12 can trigger some bystander activation but experiments using HLA-A2/HBV peptide multimers confirmed significant recovery of cytokine production by HBV-specific CD8 as early as 16 h post stimulation. This effect could not be seen in CMV-specific T cells from patients with CHB, which showed the same magnitude of response when cultured with or without IL-12. Our findings suggest that exhausted HBV-specific T cells require augmentation of signal 1 with signalling via IL-12 (signal 3) to increase effector functions. We hope that this study will contribute to inform future immunmodulatory therapies to combat chronic HBV infection.

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$CD141^+$ human migratory dendritic cells are homologous to murine CD103+ dendritic cells and excel at exogenous antigen cross-presentation

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Dendritic cells (DC) are critical regulators of immune responses. DC heterogeneity implies functional specializations with important relevance to any DC-based therapeutic strategies. Significant inroads have been made into understanding murine non-lymphoid tissue DC functions but how this translates to human DC biology is unclear. We undertook a comprehensive analysis of the human non-lymphoid tissue antigen presenting cell compartment to identify potential homologues between humans and mice.

Our dissection of the human dermis, lung and liver by multiparameter flow cytometry shows the presence of three myeloid DC subsets; CD1c+, CD14+ and a CD141+CLEC9A+ subset. CD141+ DCs migrate spontaneously, which was enhanced by XCL1, from skin explant cultures. Microarray and RQ-PCR analyses revealed equivalence between human interstitial CD141+ DCs with murine interstitial CD103+ DCs.

Dermal CD141+ DCs were superior to CD1c+ and CD14+ DCs in activating alloreactive T cell proliferation. All three interstitial DC subsets internalized exogenous soluble Hepatitis B surface antigen (HBsAg) but CD141+ DCs were vastly superior at cross-presenting HBsAg to activate HLA-A2*01 s183-91 restricted $CD8^+$ T cell clones.

Blood CD141+ DCs were not in active DNA replication but up to 1.5% of dermal CD141+ DCs were in S/G2/M phase. Blood CD141+ DCs are related to interstitial CD141+ DCs by the acquisition of a number of surface markers suggesting that interstitial CD141+ DCs are likely to derive from circulating blood precursors.

Our findings describe a human interstitial DC subset homologous to murine $CD103^+$ DCs with potent cross-presenting capacity that can be potentially targeted for anti-viral and anti-tumour responses.

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Elevated serum IL-10 levels are associated with T cell apoptosis in acute dengue infection

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The occurrence of dengue haemorrhagic fever (DHF) is thought to result from a complex interplay between the virus, host genetic makeup and host immune factors. We set out to determine possible cytokines that may contribute to the development of severe dengue infection.

We analyzed lymphocyte subsets and Annexin V expression of lymphocytes and determined the serum cytokine levels using multiplex bead array analysis in 112 adult patients with DHF from Sri Lanka. Of the 112 DHF patients' studieds, 29 developed shock.

We found that severe dengue was associated with a reduction of all T cell subsets and an expansion of the $CD56^{bright}$ NK cell population. Serum IL-10 levels positively (Spearman's $R = 0.35$) and significantly ($P = 0.02$) correlated with T cell apoptosis, while IL-10 levels inversely correlated with T cell numbers (Spearman's $R = -0.31$, $P = 0.04$). TGFß showed a very significant ($P < 0.0001$) and positive correlation (Spearman's $R = 0.65$) with platelet counts, suggesting that TGFß and IL-10 have differential roles during acute severe dengue infection.

In conclusion high serum IL-10 in patients with DHF, associate with T cell apoptosis and may contribute to the pathogenesis of severe disease.

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The expression of CD25, CD11b, MHC-II, SWC1, SWC7, CD45RA and CD45RC on porcine $\chi\delta$ T lymphocytes isolated from different organs

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The expression of selected molecules was chosen to study porcine $\chi\delta$T lymphocytes and their CD2/CD8 subsets. Virgin immune system of germ-free piglets was compared with young and adult conventional pigs. $CD25^+$ $\chi\delta$T cells are most abundant in mucosa and their amounts increase with age. On the other hand, CD11b expression on $\chi\delta$T cells can be found in every studied tissue apart from thymus and their amounts decrease with age and colonization. Increase in $CD25^+$ $\chi\delta$T cells can be mainly ascribed to $CD2^+CD8^-$ subset while decrease in CD11b can be ascribed to $CD2^-CD8^-$ subset. Activation experiments revealed that PMA caused increase in both $CD25^+$ and CD11b $\chi\delta$T cells while IL-2 stimulation influence only CD25 expression. Adult animals are generally less sensitive to activation than germ-free animals and activation primarily influenced $CD2^-CD8^-$ subset. Investigation of other molecules revealed age dependency for expression of SWC1, CD45RA/RC and MHC-II, but not SWC7. On the other hand, while the proportion of $SWC1^+$ and $SWC7^+$ $\chi\delta$T cells could increase with stimulation, $MHC\text{-}II^+$ and $CD45RA/RC^+$ could not. Our findings indicate that CD25 could represent activation marker for $\chi\delta$T cells while CD11b and probably SWC1 may be considered as possible developmental markers. The role of MHC-II and CD45RA/RC is unclear although their expression profile is age-dependent. Significant is absence of CD11b and lower occurrence of CD25 and CD45RA/RC expression in the thymus, which is the site of $\chi\delta$T cell development. This work was supported by grants GA CR 524/07/0087 and P502/10/0038, GA UK 151-43-251119 and MSMT ME09089.

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Making tumour-associated antigens more immunogenic; lessons from positional scanning synthetic combinatorial libraries

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The melanoma associated antigen (MAGE) family of proteins are overexpressed in various tumours types. This feature makes MAGE-derived peptides attractive targets for therapeutic and prophylactic vaccination strategies. Unfortunately, most-to-all tumour-associated antigens (TAAs) exhibit low T cell immunogenicity, limiting their use in vaccine development. Positional scanning synthetic combinatorial libraries (PS-SCLs) have the potential to overcome this limitation by 'tweaking' the affinity between the TAA and the T cell receptor (TCR). In this study, we generated a T cell clone specific for the MAGE epitope $_{255}$YLEYRQVPG$_{264}$ and scanned the clone across a PS-SCL array. The array identified several residues on several backbone positions which could improve T cell engagement. Substituting these suggested residues into the native peptide produced a set of super-agonists which improved T cell recognition 10 000-fold. These data have important implications for cancer vaccine development.

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Assessment of poly (Lactic:glycolic acid) nanoparticles as an antigen delivery system for cattle

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Poly(lactic-co-glycolic acid) (PLGA) is a biologically inert co-polymer which has been used to deliver a sustained release of antigen in the form micro- and nanoparticles. Encapsulation of antigen has been shown simulate a durable type 1 immune response including CD8 T cell responses through access to cross presentation pathways. Here we evaluated the use of polymeric nanoparticles as a means of delivering viral antigens to cattle. Particles synthesised were approximately 300 nm in diameter, possessed a negative surface charge and were efficiently taken up by bovine dendritic cells. Antigens could be loaded into the particles using a double emulsion method and loading was found to be approximately 30% efficient. Using EDC linking, antigen could additionally be coated to the surface of particles with an efficiency of 75%. Particle pulsed DC were able to stimulate recall T cell responses when using bovine viral diarrhoea virus (BVDV) antigens. The optimal particle was evaluated *in vivo* in a challenge model. Calves were divided into three groups ($n = 6$) which received either a commercial BVDV vaccine, control nanoparticles containing OVA and poly (I:C) or BVDV antigens which were loaded with poly (I:C) into and coated onto the particles. Antibody and T cell responses induced by each of the formulations were analysed and the ability to confer protection evaluated.

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The role of Zap70 in naïve CD8 T cell survival

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TCR signalling is crucial to both T cell development and peripheral homeostasis. The survival of naïve T cells in the periphery is also thought to depend on constitutive TCR signalling. The tyrosine kinase, Zap70 is essential for TCR signalling in T cells. The nature of this TCR dependent survival signal remains controversial. The aim of this project is to investigate the role of Zap70 in the transduction of survival signals in naïve T cells. Using mice that conditionally express Zap70 by means of a tetracycline-inducible system, we found that Zap70 is absolutely required for the maintenance of naïve T cells in the periphery. Loss of Zap70 resulted in a dramatic reduction in mature naive CD8 T cells in the blood and a loss of T cell development in the thymus, compared to control mice. This survival defect could not be accounted for by cell-intrinsic differences in IL-7R or Bcl-2 expression. We are currently examining the mechanism of this survival defect using RNA sequencing by comparing transciptomes from CD8 T cells expressing low Zap70 to control transcriptomes.

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Transcripts encoding ligands of the epidermal growth factor receptor are differentially expressed by $CD4^+$ T cell subsets, dependent on antigen experience

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Amphiregulin (Areg) is one of seven ligands for the epidermal growth factor (EGF) receptor, synthesised as transmembrane precursors prior to cleavage by one of a family of metalloproteinases, including the disintegrin and metalloproteinase domain-containing protein 17 (ADAM17). Several of the ligands are thought to be expressed by T cells, although little is known about the subsets of T cells involved or the role of the ligands in the adaptive immune system.

Microarray (GLYCOv4; Affymetrix) investigation of murine naïve (Tn), memory (Tm) and natural regulatory (Treg) $CD4^+$ T cells revealed a greater abundance of Areg transcripts in the latter two subsets [Treg/Tm: mean 3.15-fold difference, $P = 6 \times 10^{-7}$, false detection rate (FDR) = 4×10^{-5}; Tm/Tn: mean 2.31-fold difference, $P = 5 \times 10^{-4}$, FDR = 5×10^{-3} ($n = 4$)]. These differences were confirmed by quantitative (q) RT-PCR using specific TaqMan probes [Treg/Tm: 3.64 ± 1.84 (relative expression ± standard deviation); Tm/Tn: 16.41 ± 3.9 ($n = 5$)].

A qRT-PCR screen of the other ligands, the EGF receptor and ADAM17 was undertaken. Of the detectable transcripts, mRNA encoding heparin-binding EGF-like ligand (HB-EGF) was more highly expressed by the memory and regulatory $CD4^+$ T cell populations than their naïve counterparts [Treg/Tm: 4.65 ± 0.98; Tm/Tn: 3.02 ± 2.58 ($n = 4$)]. The other transcripts were expressed more evenly across the cell populations (Treg/Tm: betacellulin, 0.56 ± 0.56; EGF, 1.01 ± 0.46; ADAM17, 1.02 ± 0.49; Tm/Tn: betacellulin, 1.05 ± 0.63; EGF, 0.99 ± 0.39; ADAM17, 1.57 ± 0.68). Current studies are exploring protein expression of Areg and HB-EGF by the various T cell subsets. We propose that differential T cell expression of these ligands is related to antigen experience and has functional significance.

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Loss of Nrf2 has distinct effects on NF-κB and MAPK signalling in dendritic cells

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Dendritic cells (DCs) are antigen-presenting cells capable of stimulating T cells, producing a primary immune response. DCs depend on extracellular stimuli, intracellular signalling, including activation of transcriptional factors for their differentiation, maturation and antigen presentation. MAPK is one of the signalling pathways which are highly conserved in DCs whilst NF-kB is a transcriptional factor which governs many biological changes in DCs including co-stimulatory marker expression. Studies have shown that intracellular redox influences these signalling pathways and expression of Nrf2, a redox sensitive transcription factor, has been shown to affect many DC functions.

We examined the role of Nrf2 in DC biology with regards to NF-kB activity and MAPK signalling using bone-marrow derived DCs from Nrf2 deficient mice. We found that $Nrf2^{-/-}$ DCs have higher basal NF-κB activity than $Nrf2^{+/+}$ DCs and there are kinetic differences in IκB alpha degradation in response to TLR stimulation. Moreover ERK1/2, p38, JNK and c-Jun in $Nrf2^{-/-}$ DCs are basally hyperphosphorylated and again we have observed kinetic differences in phosphorylation upon TLR stimulation.

Our findings suggest that Nrf2 is involved in maintaining the integrity of intracellular signalling pathways in DCs.

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Susceptibility of T cells to death ligand-mediated deletion in the liver of chronic hepatitis B virus (CHB) infected patients

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Persistent infection with HBV is associated with a number of T cell defects culminating in marked depletion of the HBV-specific T cell responses. We hypothesised that profoundly dysregulated T cells directed against HBV may become susceptible to autologous killing via the TRAIL pathway. We found an increase in the expression of TRAIL-R2, transducing apoptotic signals on TRAIL binding, on peripheral T cells from CHB patients compared to healthy controls ($P < 0.05$). TRAIL-R2 was further upregulated on intrahepatic compared to peripheral T cells (mean ± SEM, 20 ± 2.9 versus 1.4 ± 0.6, $P < 0.001$) directly *ex vivo* in 14 CHB individuals with paired samples. TRAIL-R2 was not increased on intrahepatic T cells from controls including HCV patients with similar levels of activation. TRAIL-R2 was found to be substantially higher on intrahepatic HBV-specific T cells identified following overnight incubation with overlapping peptides (OLP) spanning the core protein of HBV. TRAIL blockade increased recovery of HBV-specific T cells after overnight culture in four out of nine patients. The incomplete recovery of virus-specific T cell responses suggests that some responses are committed to their apoptotic fate and no longer amenable to rescue at the receptor level. This is supported by our preliminary data showing high levels of caspase-8 in TRAIL-R2-positive intrahepatic T cells from CHB patients *ex vivo*. In summary, we provide initial evidence of a new pathway whereby upregulation of TRAIL-R2 on HBV-specific T cells in the HBV infected liver may render them susceptible to ligand-mediated deletion.

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MAIT cells induce proinflammatory cytokine production in monocytes through the secretion of granzymes A and K

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Mucosal-associated invariant T (MAIT) cells are a population of CD161++CD8 T-cells expressing the semi-invariant T-cell receptor iVα7.2-Jα33, but their functions remain unclear. In this study we examine the unique expression profile of cytolytic molecules in these cells. We report that MAIT cells are characterised by a lack of granzyme B (GrB) and perforin, key granule proteins required for efficient cytotoxic activity, but highly express granzyme A (GrA; 99.5% $P < 0.0001$) and granzyme K (GrK; 95.1% $P < 0.0001$) compared to CD161− CD8 T-cells. Furthermore, we found a dramatic increase of GrA ($P = 0.0007$) and GrB ($P < 0.001$) in CD8 T-cells in HIV patients compared to healthy donors, while GrK was found to be reduced in HIV ($P = 0.0046$) and HCV patients ($P = 0.0282$), resulting from the loss of MAIT cells from the periphery. GrA and GrK are trypsin-like serine proteases originally believed to function exclusively as pro-apoptotic proteases, but recent studies suggest that these molecules may possess proinflammatory functions. We therefore tested the hypothesis that granzyme release from MAIT cells induces proinflammatory cytokine production from target cells. With a novel cytotoxicity assay called the APE assay, we examined the ability of MAIT cells to kill target cells, and demonstrate that extracellular GrK induces secretion of IL-1β, TNFα, and IL-6 in a time-dependent manner from monocytes. Together with their ability to secrete IL-17 and gut-liver homing characteristics, GrA and GrK expression most likely participate in the antimicrobial properties of MAIT cells and may contribute to tissue inflammation in diseases such as chronic HCV.

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Immune memory to *Neisseria meningitidis* protein antigens in humans

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Neisseria meningitidis is a common cause of bacterial meningitis and septicaemia in children and young adults. Effective vaccines are available for serogroups A, C, W and Y but there is currently no universal vaccine against serogroup B meningococci. Serogroup B is responsible for the majority of meningococcal cases in the UK. Research has indicated that meningococcal outer membrane proteins (OMPs) could potentially be used as vaccines against serogroup B. These OMPs include the iron regulated protein FetA and the porin PorA. We aimed to study the memory B cell response to these protein antigens in humans.

Human adenotonsillar cells were stimulated with concentrated meningococcal culture supernatants (CCSs) produced from log phase cultures of five *N. meningitidis* isolates. The frequency of antigen-specific antibody-secreting cells to PorA and FetA were then determined using ELIspot assays. Results show that some individuals had developed memory B cells to meningococcal PorA and FetA proteins. This memory appeared to be strain specific and is likely to be due to previous meningococcal exposure. In order to identify immunogenic meningococcal proteins, meningococcal CCSs were immunoblotted with human serum. Immunoblots indicated that some individuals had developed a natural immune response to *N. meningitidis*. Common and variable immunogenic meningococcal proteins were detected between different meningococcal CCSs.

The preliminary findings of this study suggest that natural immunological memory responses to *N. meningitidis* antigens develop in some individuals. PorA and FetA may represent two important antigens in the development of this response and therefore may be suitable vaccine candidates.

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Expression of the TGF-beta-activating integrin alphaVbeta8 on dendritic cells is essential for the suppression of systemic immune responses during oral tolerance

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Regulatory T cells (Tregs) play a pivotal role in mediating tolerance induced at mucosal sites following exposure to ingested antigens. In particular the TGF-beta dependent conversion of T-cells to inducible Foxp3+ Tregs (iTregs) helps to avoid disproportionate negative immune responses against food antigens. TGF-beta is produced as a latent cytokine that must be activated to function. We have recently demonstrated that a specific dendritic cell (DC) subset of the gut is specialized to induce Foxp3+ iTregs due to an enhanced ability to activate TGF-beta via increased expression of the integrin alphaVbeta8. However, the role of this pathway in regulating systemic immune responses to ingested antigens, which is a hallmark of oral tolerance, is unknown.

Here we show that expression of the TGF-beta-activating integrin alphaVbeta8 on DCs is essential for the induction of oral tolerance. DC-specific alphaVbeta8 null mice are completely susceptible to the development of a severe delayed type hypersensitivity response even after oral administration of OVA antigen. We therefore investigated if these mice had a reduced ability to induce Tregs *in vivo*. Indeed, the Foxp3+ Tregs of DC specific alphaVbeta8 null mice had a reduction in the Helios negative iTreg subset within the lamina propria. These data correlate with our previous findings that transferred CD4+ OT-II cells are impeded from becoming iTregs following oral feeding of OVA antigen in DC-specific alphaVbeta8 null mice. These results identify the importance of integrin-mediated TGF-beta activation not only in promoting a tolerogenic environment in the gut, but also in governing systemic immune responses.

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Virus transformed human B cells present DEC-205-targeted antigens better to T cells than dendritic cells

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DEC-205 is a type I transmembrane multilectin receptor that has been explored for targeting vaccine antigens to dendritic cells (DCs), in order to improve processing and presentation on MHC I and MHC II molecules. As DEC-205 is predominantly expressed on DCs, previous studies primarily focused on processing of DEC-205-targeted antigens by this potent antigen presenting cell type. Here we show that Epstein Barr virus (EBV) transformed lymphoblastoid B cell lines (LCLs) not only express DEC-205 at similar levels to DCs, but also efficiently present targeted EBV nuclear antigen 1 (EBNA1) and EBV-latent membrane protein 1 (LMP1) to EBNA1- and LMP1-specific $CD4^+$ and $CD8^+$ T cell clones *in vitro*. However, targeting of antigens to DEC-205 leads to more efficient MHC class II than class I loading. Surprisingly, targeting of antigens to DEC-205 of LCLs stimulates T cells more efficiently *in vitro* than targeting to DEC-205 on DCs. While LCLs internalized DEC-205 targeted antigens less efficiently than DCs, they maintained them for longer periods without degradation and delivered them to endosomal compartments that receive also B cell receptor targeted proteins. This could facilitate prolonged T cell stimulation and efficient MHC class II loading. These studies suggest that B cells, activated by virus transformation, can contribute to T cell stimulation after DEC-205 targeting of antigens during vaccination.

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The immunological effects of co-administering diphtheria, tetanus, pertussis combined vaccine with measles vaccine to 9 month old Gambian infants

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Introduction and aims: Many commonly used vaccines were developed empirically, and there is surprisingly poor understanding of how they work. There is increasing evidence that aside from vaccine-specific effects, vaccines have non-specific effects (NSE) leading to altered morbidity and mortality from other infections. Broadly, live vaccines such as measles vaccine (MV) are beneficial, whereas inactivated vaccines such as diphtheria, tetanus, pertussis vaccine (DTP) may be deleterious. When live and inactivated vaccines are administered simultaneously the beneficial NSE of the live vaccine are lost. Females are generally more susceptible to these NSE than males. This study aimed to investigate the immunological mechanisms behind these observations.

Methods: Three hundred and three children were randomised to one of three vaccine groups; Group 1: MV at 9 months; Group 2 DTP + MV at 9 months; Group 3 DTP at 9 months. Children were bled at 9 months and 4 weeks later. Females and males were randomized separately. Immunological assays included overnight whole blood cultures, flow cytometry for intracellular cytokines, β-2 microglobulin (β2M) ELISA, measles and DTP Ab assays, and whole human transcriptome microarray analysis from whole blood.

Results: Distinct male/female differences were found in β2M levels, cytokines in culture supernatants, and the transcriptome profile, with females generally being more immune activated and pro-inflammatory than males. Vaccine antibody levels were unaffected by combining the vaccines.

Conclusion: This study shows evidence of sex differences in response to vaccines, and that combining MV with DTP alters the immune profile.

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Phylogenetic analysis of the evolutionary conservation of the Foxp gene sub-family within the Animalia Kingdom

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The Foxp gene sub-family forms a clade of the Fox family and comprises four members, Foxp1 to 4. All members share a common 100 amino-acid Forkhead domain. Expression of Foxp1, Foxp2 and Foxp4 is required for development in vertebrates: loss of function is associated with tumour growth and loss of Foxp2 has been implicated in speech defects in humans. Foxp3 has an essential role in the maintenance of peripheral tolerance and is expressed by a subset of T cells - regulatory T cells (Tregs) – in many species. Loss of Tregs is associated with autoimmune disease. The prevalence of the Foxp gene sub-family across various phyla suggests a high level of functional conservation between species. We hypothesised that owing to the fundamental importance of these members there would be conservation of structure at both the protein and gene level. A SMART search was used to identify Foxp members in 52 different species, based on a shared structure comprising a Forkhead, C2H2 Zinc finger and a Leucine Zipper domain. Preliminary results suggest that Foxp2 and Foxp3 are the most evolutionarily divergent members. Intriguingly, Foxp3 appears to be absent from the Aves class even though Tregs have been described in this species. Further work is ongoing to reconcile the species tree with the Foxp sub-family gene tree to characterise the events that led to the evolution of the respective family members.

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Immunological memory response to seasonal and pandemic H1N1 influenza in children and adults

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Influenza kills millions of people worldwide each year. The local immunity may be crucial in protection against severe disease. It is important to know whether and how natural immunity develops in humans due to previous exposure and whether such immunity provides any cross protection against future pandemic influenza. We aim to investigate the memory T and B cell response in humans to both seasonal and pandemic H1N1 influenza.

Methods: Adenotonsillar tissues and peripheral blood were obtained from children and adults. Memory T and B cell responses to haemagglutinin (HA) of both seasonal and pandemic H1N1 were analysed by T cell proliferation assay and Elispot assay respectively. Antibodies were analysed by ELISA.

Memory T cell proliferation was shown in adenoidal MNC and PBMC to seasonal H1N1 HA antigen in most subjects tested ($P < 0.01$). Memory T cell response to the pandemic H1N1 HA was also demonstrated in some subjects. A stronger memory T cell response to seasonal H1N1 HA than to the pandemic HA was shown. There seem to be a positive correlation between the T cell response to seasonal and pandemic H1N1 HA. Significant memory B cell responses to both seasonal and pandemic H1N1 HA were also observed. A similar correlation was also seen in serum antibody levels between the seasonal and pandemic H1N1.

Conclusion: Significant memory T and B cell responses to both seasonal and pandemic H1N1 HA are developed after natural infection in children and adults. The natural immunity to the seasonal H1N1 may cross-reactive with the pandemic H1N1 flu.

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The adoptive transfer of pulsed BMDC enhances specific immune responses and provides protection against lethal anthrax challenge

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Dendritic cells are potent activators of the immune system and have a key role in linking innate and adaptive immune responses. In the current study we have used *Bacillus anthracis* as a model to develop an adoptive transfer strategy, using pulsed Bone Marrow Dendritic Cells, to provide protection against infectious agents. Recombinant Protective Antigen (rPA) formulated with alum provides a high titre antibody response to rPA, a detectable T-cell response and protection against lethal anthrax challenge. BMDC pre-pulsed with heat-killed *B. anthracis*, rPA and CpG have upregulated expression of CD80, CD86 and MHC-II, and by 7 days post transfusion have induced a specific T cell response in recipient mice. When rPA in alum is co-delivered with pulsed BMDC we see enhanced T cell and antibody responses compared to rPA and alum alone. Mice vaccinated with a sub-optimal rPA and alum dose together with pulsed BMDC have 100% survival following lethal *B. anthracis* challenge, compared to 80% for rPA and alum vaccinated control mice and 40% for mice receiving pulsed BMDC only. In the future, we plan to advance this strategy to include other infectious agents.

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Costimulatory receptors CD27 and 4-1BB as targets for the promotion of CD8 T-cell responses: 4-1BB signals during priming preferentially enhance generation of memory T cells

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CD27 and 4-1BB signals allow for optimal CD8 T cell expansion during infection. Furthermore, CD27 or 4-1BB triggering can substantially enhance CD8 T cell responses elicited by non-inflammatory antigens. Consequently, agonist CD27 and 4-1BB monoclonal antibodies are currently being developed for clinical use as adjuvants to promote cellular immunity against cancer. Whether engagement of CD27 or 4-1BB instigate a similar T cell differentiation programme is unknown. To better understand how CD27 and 4-1BB influence CD8 T cell differentiation we analysed antigen-specific CD8 T cells following immunisation with non-inflammatory antigen and either CD27 or 4-1BB agonists. While both types of agonists were similarly effective in enhancing the magnitude of the primary response, 4-1BB stimulation generated significantly more memory CD8 T cells. Further experiments demonstrated that the effect of 4-1BB stimulation on memory T cell generation occurred at an early stage during priming and differences between CD27 and 4-1BB stimulated T cells were identified as early as 3 days after immunisation. A striking difference between the CD27 and 4-1BB stimulated T cells was the enhanced frequency of IL-2 producing CD8 T cells and prolonged expression of the IL-2 receptor (CD25) in the CD27 arm. A more comprehensive analysis of the differences between CD27 and 4-1BB stimulated T cells was obtained by global gene expression profiling. A subset of NF-kB target genes was found to be enriched in the CD27 stimulated T cells demonstrating previously unappreciated differential regulation of NF-kB responsive genes by signalling through different members of the TNF receptor superfamily.

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T follicular helper cells in peripheral and gut associated lymphoid tissue

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Plasma cells produced in the peripheral systemic immune system and the gut have different features. Most plasma cells generated in peripheral lymphoid tissues secrete IgG that tends to be highly antigen specific. In contrast, plasma cells generated in the gut mostly secrete IgA antibodies that can be poly-specific or autoreactive.

T follicular helper cells (TFH) reside in germinal centres and aid clonal expansion of B cells and the production of higher affinity plasma cells. The aim of this study is to investigate the possibility that subsets of TFH in peripheral and gut associated lymphoid tissue (GALT) may account for some of the differences in properties of plasma cells and the antibodies they secrete.

TFH tend to be CD4 T cells that may also express CXCR5, PD-1 and CD57. When tissue sections of peripheral lymphoid tissues and GALT were studied by immunohistochemistry, it was apparent that there is a higher density of TFH in GALT compared to peripheral lymphoid tissue. This may reduce the threshold for B cell survival during the affinity maturation process, permitting the production of less stringently selected antibodies. Also a higher percentage of TFH in GALT lack CD57. Therefore cytokine production by, and CD40L expression on, CD57+ and CD57− TFH has been characterised by flow cytometry and will be described.

TFH are important in the generation of plasma cells. Therefore differences in TFH between gut and the periphery could contribute to differences in the properties of plasma cells and the antibodies they secrete.

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Genetic approaches to the study the role of retinoic acid in the regulation of B cell immunity in the gut

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Retinoic acid (RA) plays an important role in the balance of inflammation and tolerance on T cells. Furthermore, it has been demonstrated that induces IgA isotype switching on B cells *in vitro*. However, it is unclear whether RA has a direct effect on B cells to induce IgA isotype switching *in vivo*. We have been able to reproduce similar data using B cells that express a dominant negative for the retinoic acid receptor (dnRAR). In this model, we can quantify the levels of IgA produced for Plasma cells (PCs) by Elisa and the percentage of IgA PCs by flow cytometry. After 4 days of culture with 100 nM of RA, dnRAR B cells are unable to differentiate to IgA PCs. In addition, they produced very low levels of IgA compare with control. On the other hand, the levels of IgA from intestine lavage and number of IgA B cells in the intestine are similar between dnRAR and control mice, suggesting that RA does not play a direct role on B cells in the differentiation of IgA PCs *in vivo*. We hypothesize that RA affects indirectly the B cell compartment to generate the differentiation to IgA PCs in the small intestine.

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Arf6 – a potential player in T cell biology

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The ADP ribosylation factor (Arf) family of GTPases comprises of small monomeric proteins. Among the three known subclasses, the highly expressed Arf6 is functionally distinct, and is implicated ubiquitously in membrane trafficking, endocytosis, exocytosis in various cells and invasive activities of cancerous cells. There is little known about the role of Arf6 towards the mechanisms of T cell signal transduction and Arf6 null mice are embryonically lethal which limits the role of KO models for investigating its regulatory mechanisms. In the present piece of work, it was found that disruption of Arf6 activity leads to impairment of downstream signalling events underlying TCR signalling. This includes cell cycle progression, proliferation and T cell expansion and IL-2 production. These effects are mediated by cytohesins-the regulating factors for Arf6. Moreover, it was found that Arf6 is specifically regulated in actively dividing immune cells like thymocytes, splenocytes, effector T cells. Also, regulatory T cells express high levels of Arf6 and secrete high levels of suppressive cytokine IL-10, indicating a potential role of Arf6 in inhibition of activation of T cells. The data provides direct evidence that Arf6 constitutes an important component of TCR signalling in primary T lymphocytes. To study the mechanistic regulation of Arf6, *in vivo* model would prove to be an important asset. For this, a targeting vector is developed to conditionally delete Arf6 in T cells to elucidate the exact role of Arf6 in an *in vivo* setting. This model will expand our understanding of human disease associated with immune dysregulation.

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Natural variation of FcgammaRIIb expression controls the germinal centre reaction, affinity maturation and autoimmunity

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The inhibitory receptor FcgammaRIIb plays a crucial part in the control of antibody-mediated immune response. In humans, naturally occurring polymorphisms altering the function or expression of FcgammaRIIB have been associated with increased susceptibility to autoimmune diseases. Mice deficient for FcgammaRIIb are more susceptible to autoimmunity but are protected against some infections. Several polymorphisms in the promoter region of *Fcgr2b* have been described in mouse strains prone to autoimmunity. These polymorphisms are associated with lower expression of FcgammaRIIb on activated B cells. Interestingly, most wild mice from the *Mus musculus* genus bear the *Fcgr2b* promoter polymorphisms present in autoimmune strains suggesting that they have been conserved during evolution. We generated a knock-in mouse were the FcgammaRIIb promoter was from wild mice and NZB origin on a C57BL/6 background to analyze the effect of naturally occurring polymorphisms on the immune regulation. Basal expression of FcgammaRIIb was normal in these mice but activation-induced up-regulation of FcgammaRIIb expression was completely abrogated on germinal centre B cells. This reduced level of FcgRIIb expression was associated with increased number of germinal centre B cells and antigen-specific plasma cells. Moreover, affinity maturation in germinal centre B cell and serum IgG was increased. We also demonstrated that this germinal centre-specific reduction of FcgammaRIIb expression was associated with the development of auto-antibodies and with increased severity in collagen-induced arthritis. Altogether, our results show that subtle variation of FcgammaRIIb expression controls the size and quality of the germinal centre reaction and contributes to the development of autoimmunity.

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Predominance of heterosubtypic IFN-γ-only-secreting effector memory T cells in pandemic H1N1 naive adults

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The 2009/2010 H1N1 (pH1N1) pandemic highlighted the need for vaccines conferring heterosubtypic immunity against antigenically shifted influenza strains. Cross-reactive T-cells to conserved viral proteins are strong candidates for mediating heterosubtypic immunity and T-cell functional subsets defined by cytokine-secretion patterns are important in anti-viral protective immunity, vaccine development and categorising models of viral infections based on antigen load and exposure. However, little is known about the prevalence, frequency and phenotype of heterosubtypic cytokine-secreting functional T-cell subsets to pH1N1. To assess this, pH1N1 sero-negative healthy adults were recruited and single-cell cytokine-secretion profiles for IFN-γ and IL-2 specific for PB1, M1 and NP proteins of pH1N1 or live virus were enumerated by fluorescence-immunospot and further characterised by flow-cytometry.

Heterosubtypic T-cells to pH1N1 core proteins were detected in a high proportion (30 of 33, 90%) of individuals. The magnitude and prevalence of IFN-γ-only-secreting T-cells to pH1N1 was significantly higher than IL-2-only-secreting T-cells. These heterosubtypic IFN-γ-only-secreting T-cells were predominantly CCR7-CD45RA- effector-memory phenotype, expressed tissue-homing receptor CXCR3 and degranulation marker CD107.

This surprisingly high prevalence of pre-existing cross-reactive CD8+ IFN-γ-only-secreting effector-memory T-cells with cytotoxic and lung-homing potential may partly explain the lessened severity of the pandemic in young adults. The unexpected predominance of IFN-γ-only-secreting memory T-cells >6 months after the last exposure to influenza, suggests that the functional T-cell signatures to a recurrent acute viral infection such as influenza in humans, is distinct from the signature of an acute cleared infection or chronic persistent infection.

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CD_4 T cell response to MVA85A vaccination against *Mycobacterium tuberculosis* in healthy HIV-infected adults

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Control of the TB epidemic is a global health priority and is only likely to be achieved through vaccination. Due to the critical overlap with the HIV epidemic and TB infection any effective TB vaccine regimen must be safe in HIV-infected individuals. An open-label Phase I trial in 20 UK volunteers recently evaluated the safety and immunogenicity of a leading candidate TB vaccine, MVA85A, in healthy, HIV-infected adults.

Various clinical and immunological parameters assessed vaccine safety, immunological effects and interaction with HIV, in particular the risk of preferential HIV infection of vaccine-induced activated CD4 T cells.

Laboratory-measured outcomes included CD4 count and HIV RNA load; qPCR analysis of integrated HIV DNA transcript in CD4 T cells; surface expression of HIV co-receptor molecules on CD4 T cells; and flow cytometric, ELISpot and ELISA analysis of chemokine and cytokine production.

These data showed that MVA85A, a leading candidate TB vaccine, is well tolerated in healthy HIV-infected people. MVA85A induced a similar immune response profile to that seen in HIV-uninfected people, with an observable dose-response effect. In this small study there was no evidence to suggest MVA85A vaccination lead to widespread preferential infection of vaccine-induced CD4 T cell populations.

These data are encouraging for this vaccine candidate, as well as the wider TB and HIV vaccine research communities. They merit further study in larger trials in endemic populations.

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Analysis of antigenic diversity in the intracellular parasite *Theileria parva* using 454 DNA sequencing technology

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It has been shown that CD8 T cells play an important role in immunity to the intracellular protozoan parasite *Theileria parva*. A number of antigens have been identified that are recognized by bovine parasite-specific CD8 T cells, some of which are highly polymorphic. After immunisation with live parasites, solid immunity is seen against the homologous parasite, but immunity against heterologous parasites is variable. A high throughput sequencing approach has been used to investigate the antigenic diversity in *T. parva*, both at the population level and within individual isolates. Amplicons of the regions of the antigen genes encoding defined epitopes have been generated by PCR for sequencing using 454 technology. Initial studies have focused on analyses of diversity in a live *T. parva* vaccine (the Muguga cocktail), which incorporates three cattle-derived parasite stocks, and in samples obtained from African Buffalo, the natural wildlife reservoir. Initial results indicate that parasites in individual buffalo exhibit extensive antigenic diversity, whereas the components of the live vaccine have very limited diversity. The establishment of an antigenic profile for the vaccine will potentially allow the development of assays to assess the consistency of different vaccine batches and will aid in the phenotypic analysis of vaccine- breakthrough parasite strains.

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Immune responses in acute and chronic HCV genotype 3a infection detected by two distinct techniques

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Detailed analysis of T-cell immunity across all Hepatitis C (HCV) genotypes is important for T-cell vaccine development. HCV subtype 3a (gt3a) is now the commonest infecting genotype in the United Kingdom; however, data regarding T-cell antigenic targets of this subtype is very limited.
Methods: We used two different approaches to identify T-cell targets in acute and chronic HCV gt3a infection: (i) overlapping peptides (whole viral genome, 15–18 amino-acids (AA), overlapping by 11 AA), generated based on a consensus sequence. (ii) A novel, sequence-led approach using 46 wild-type and variant peptides (non-structural proteins, 9–10 AA) corresponding to putative HLA class-I restricted epitopes. These were identified through association of HLA-type with polymorphic viral genomic sites following sequence analysis in 136 gt3a patients. T-cell responses were measured by Interferon-γ-ELISpot assays in 84 gt3a-infected patients (10 acute, 74 chronic). Responses were confirmed and further characterized using ICCS following short-term cell lines.
Results: In acute patients, 10 T-cell epitopes were identified using overlapping peptides, predominantly targeting non-structural proteins. Four additional T-cell targets were detected using the HLA-restricted peptides. In chronic infection, T-cell responses were detectable in 35/74 patients using overlapping peptides, mainly targeting core (27/35) and NS3 (12/35), and in 8/51 patients using HLA-restricted peptides.
Conclusions: Using two parallel methodologies we have identified multiple new T-cell epitopes in HCV gt3a infection. Overall, the two methodologies are complementary and identify distinct T-cell targets. In acute infection, non-structural proteins are dominant $CD8^+$ T cell targets. $CD4^+$ T-cell responses to core develop once chronic infection is established.

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CTLA-4 controls the thymic development of conventional and regulatory T cells

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The generation of T cells in the thymus depends on an interplay of T cell receptor affinity, the quality and quantity of specific antigen, locally produced chemokines and cytokines and the availability of co-stimulatory signals. The main co-stimulatory molecule, CD28, is generally accepted to augment negative selection of conventional T (Tconv) cells as well as promote the generation of $FoxP3^+$ regulatory T (Treg) cells. The role of its antagonistic homologue CTLA-4, however, remains a topic of debate. Several groups have previously suggested that CTLA-4 inhibits negative selection of Tconv cells. More recently, we demonstrated that in the Tg4 mouse model, carrying a transgenic TCR specific for MBP peptide Ac1-9, generation of $FoxP3^+$ Treg cells is enhanced in the absence of CTLA-4. We hypothesized that the deviation in thymic selection in the absence of CTLA-4 resulted from an effect on TCR rearrangement during thymic development. Here we show that CTLA-4 expression in the thymus drastically alters the TCR alpha chain repertoire; this changes TCR avidity and skews the development of both Tconv and Treg cells.

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Antibiotic administration changes the balance of effector and regulatory T-cells in the neonatal intestine

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Numerous studies suggest that antibiotic administration in early childhood is associated with an increase in the incidence of allergic disease, but the related mechanisms are difficult to study in human infants. The similarities in physiology and nutritional requirements between pigs and humans suggest that pigs can be good models for studying these effects.

To address the effect of antibiotic administration in the regulation of immune responses we have used fluorescence immunohistology to investigate $CD4^+$ $CD25^+$ $FoxP3^+$ regulatory T cells (Tregs) in the intestinal lamina propria of 28 day old piglets. All piglets were born naturally and spent their first 24 h with the sow on an extensive farm. At 24 h old, a subset of piglets from each litter was transferred into a high hygiene isolator where half were treated daily with antibiotics. After 28 days, piglets treated with antibiotics had significantly greater area of $CD4^+$ staining in the lamina propria of the small intestine than piglets reared either on the mother or in the isolator without antibiotics. Despite the increase in total $CD4^+$ T-cells, these antibiotic-treated piglets had fewer $CD4^+CD25^+FoxP3^+$ T-cells as a proportion of the total $CD4^+$ staining than their non-antibiotic-treated littermates. Our results suggest that antibiotics predispose to allergy by changing the effector-regulatory balance in the intestinal mucosa of the neonate.

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Natural immunity to pneumococcal pilus proteins and its protective role against pneumococcal carriage in humans

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Background: Pneumonia is the world's number one killer of children, accounting for approximately 2.6 million <5 children death each year, nearly half of them are attributable to pneumococcus. The available pneumococcal vaccines have limitations like high cost, low efficacy, and narrow serotype coverage. Pili of pneumococcus play important role in its virulence and may therefore be an effective vaccine candidate.
Aims and objectives: To investigate whether natural colonisation of pneumococcus primes for immunological memory to pili protein antigens (RrgA and RrgB) to protect against pneumococcal carriage and the potential of these proteins as mucosal vaccines in children.
Methodology: Culture of nasal swab for pneumococcal carriage; culture of immune cells with or without pneumococcal concentrated culture supernatants (CCS); and appraisal of antigen-specific helper T cell activity by flow-cytometry and memory B cell responses using ELISPOT assay; measurement of pilus protein antibodies in serum, cell culture supernatants and saliva by ELISA; and detection of specific serum antibodies by Western Blot.
Results and conclusion: Preliminary results suggest that natural immunity to RrgA protein antigen develop early in childhood, and there is an age-dependent increase in levels of anti-RrgA and RrgB IgG antibodies. In adults, levels of anti-RrgB antibody are higher than anti-RrgA. Results also show memory B cell response to both RrgA and RrgB protein antigens in some subjects. Nasopharyngeal carriage of pneumococcus is common in children whereas it is infrequent in adults. Association of T and B cell immunity to RrgA and RrgB protein antigens will be analysed in future studies.

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Crosstalk between complement and notch systems is required for normal function and regulation of Th1 responses

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Initially described as a complement-regulatory molecule, CD46 is emerging as vital co-stimulator in the induction/regulation of human Th1 effector cell responses. CD3 and CD46 coengagement promotes, in an IL-2-dependent fashion, a switch from IFN-γ^+ Th1 cells to IFN-γ^-/IL-10^+ cells with potent immunosuppressive/self-regulatory functions. However, the molecular pathways for CD46-mediated IL-10-secretion are not well understood. Similarly, although Notch receptors (Notch1 to -4) and ligands (Jagged1 and -2, Delta-like-1, -2, -3) play an acknowledged important role in Th1/Th2 lineage decisions, many aspects of their functional pathways remain puzzling. Because CD46 and Notch share intriguing features on a structural and functional level, we hypothesized on the existence of a CD46/Notch crosstalk during Th1 activation. Indeed, our results show that CD3/CD46-activated $CD4^+$ upregulate mRNA transcription/protein expression of Notch1 and -2 (but did not impact Notch3 or -4 expression) and Jagged1 and -2. Strikingly, CD3/CD46-activation is associated with strong Delta-like-1 downregulation (Delta-3 and -4 are not detectable), which is dependent on an intracellular interaction of CD46 with α-E-catenin, a molecule known to regulate γ-secretase/substrate interactions.

Importantly, inhibition of Delta-like-1 downregulation or interference of Notch signalling via anti-Notch mAb or soluble Notch impedes CD46-driven IFN-γ and IL-10 production, while the adjunction of soluble Delta-like-1, Jagged1 and CD46 proteins increased IFN-γ secretion while inhibiting the switch to IL-10^+ cells. Our data suggest that crosstalk between CD46 and Notch pathways is crucial in normal Th1 function and may offer novel molecular targets for the control of Th1 cell regulation in infection and immune homeostasis.

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TNFa induces increased formation of fat associated lymphoid clusters and lymph node-like structures in the mesenteries

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Lymphoid clusters named Fat Associated Lymphoid Clusters (FALCs) have recently been identified in the fat associated to the mesenteric vessels. These clusters contains $LIN^-CD3^-CD4^-Kit^+Sca1^+$ lymphoid cells or 'Natural Helper Cells' that can produce Th2 cytokines like IL-4, -5, -6 and -13. The natural helper cells present in FALCs have been shown to play an important function against parasite infection. However, the formation and the molecular requirement for the development of FALCs remain unclear. Here we show that natural helper cells appear around birth in the mesenteries, but clusters are formed only from the second week of life. FALC formation is independent of Rorgt, LTa, LTbR and CCR7 demonstrating that contrary to lymph node development, the development of these structures is not dependant on lymphoid tissue inducer cell recruitment and the activation of LTbR. Nevertheless, these clusters contain Lymphoid Tissue organizer like cells expressing Cxcl13 and are closely associated with the blood and lymphatic vasculature. Finally, we show that high levels of TNFa dramatically increase FALC numbers indicating the role of TNFa in promoting the formation of these structures. This effect is also associated with the emergence of several large lymph node-like structures along the mesenteries. These data suggest that inflammatory stimuli such as TNFa drive the formation of FALCs and thus could modulate the immune responses of the mesenteries.

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Generating improved HLA class II tetramers for investigating antigen specific $CD4^+$ T cells

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Background: T cell receptor (TCR) recognition of peptide epitopes presented by HLA class II (HLA-II) antigens governs $CD4^+$ T cell activation. The use of HLA-II multimers to identify antigen-specific populations has so far not replicated the success seen with HLA class I multimers. We have previously found modifying $CD4^+$ T cell epitopes by the substitution of basic residues in the C-terminus enhances T cell activation.
Aim: To explore the use of C-terminally modified epitopes to generate superior HLA-II tetramers.
Methods: TCR genes, derived from cognate T cell clones, and HLA-DR genes were cloned and then expressed in *E. coli*. Proteins were refolded *ex vivo* via denaturant dilution. $CD4^+$ T cell clones were used that recognised the universal epitope from haemagglutinin, $HA_{305-320}$ (Flu 1). Variant peptides were $HA_{Ala318Arg}$ (Flu 2) and $HA_{Thr319Arg}$ (Flu3). HLA/TCR interactions were measured by surface plasmon resonance (SPR). Tetramerised HLA-II binding to T cells was measured by flow cytometry.
Results: The affinity of HLA-DR1 presenting Flu1, Flu2, or Flu3 to cognate TCRs was measured by SPR. Substitution of Arginine into the C-terminus markedly increased the affinity of TCR binding as indicated by a fall in the K_D values from *x* to *y*. HLA-DR1 tetramers made with the three peptides were used to stain T cell clones and demonstrated a massive increase in binding with Flu 2 and Flu 3.
Conclusion: C-terminally modified HLA-II epitopes can significantly improve TCR binding and hence offers a route to generating high affinity tetramers with no loss in antigen specificity.

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Transcription and recombination of the IgH locus 3′ regulatory region (3′RR) during the maturation of B lymphocytes

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The immunoglobulin heavy chain (IgH) locus undergoes recombination (VDJ joining and class switching) and somatic hypermutation during the normal differentiation program of the B lymphoid lineage. Transcription of the regions targeted by these events is a pre-requisite to the accessibility of the locus to recombination enzymes. This transcription is conferred and regulated by several cis-regulatory elements located throughout the IgH locus. The intronic enhancer Eμ and the 3′ regulatory region (3′RR, located downstream of the constant gene cluster of the locus are thus major players of B cell development.

Attention has recently been focused on the transcription of some enhancer elements, although the function, if any, of the so-called eRNAs is still elusive. Using both RT-PCR and chromatin immunoprecipitation with an antibody against RNA polymerase II, we have demonstrated that the IgH 3′RR is actively transcribed in B cells and regulated along B cell activation by the 3'RR enhancers themselves. In addition, we show that the 3'RR is frequently target by recombination events in normal B cells, with strong implications on B cell fate.

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Whole genome expression pathway signatures after antigen stimulation: identifying diagnostic and prognostic disease markers

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We have established protocols for the *ex vivo* assessment of antigen specific responses and cellular immunity using qPCR with finger prick volumes of whole blood. Our assays to date use two reporters of IFNγ production - MIG and IP10 (MIG − Monokine -induced by IFNγ and IP10 − IFNγ induced protein 10). MIG and IP10, predominately expressed by CD14 monocytes and macrophages, are produced or expressed in high quantities in response to IFNγ antigen from triggered T cells i. e., in an antigen specific manner, thus making them excellent adjunct biomarkers. To date we have had success with analysis of T cell responses to TB, CMV and HIV. New data reveals the utility of such assays in epitope discovery using individuals exposed to diverse flaviviruses including Japanese Encephalitis virus (JEV).

These assays are to date are focused on Th1 responses secreting IFNγ, and use previously defined genes. Using whole gene expression arrays we are investigating if we can further define signature responses to specific cytokines: IFNg, IL10, IL13, IL17 to detect polarized Th1, Th2, Treg and Th17 responses respectively. We are conducting these gene expression profiles on whole blood over a time course, thus widening our screening approach to the full diversity of blood cellular populations (not just PBMCs). Ultimately the aim is to identify novel optimized diagnostic or prognostic markers for our antigen specific assays.

The results fro7m these studies will be discussed.

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Role of the TIM-3/Galectin-9 pathway in chronic hepatitis B infection

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An excess of co-inhibitory signals drives HBV-specific T cells to exhaustion. One such co-inhibitory signal is Tim-3 (T cell immunoglobulin and mucin domain-containing molecule 3), expression of which we found to be increased on global CD4 and CD8 T cells in CHB compared to healthy donors ($P = 0.0001$). Expression of Tim-3 was significantly increased on HBV-specific compared to CMV-specific CD8 T cells within the same individuals ($P = 0.001$) or in healthy controls ($P = 0.0001$). Our data showed that these Tim-3 expressing HBV-specific T cells have impaired production of IFNγ and TNFa upon peptide stimulation. Higher levels of circulating Tim-3 ligand, galectin-9, were found in the serum of patients with high levels of CHB-related inflammation (ALT > 100) compared to low level inflammation (ALT < 50) and healthy individuals ($P = 0.02$ and $P = 0.01$). Immunohistochemistry of CHB liver sections showed galectin-9 staining was concentrated in the Kupffer cells, confirmed by immunofluorescent co-staining with CD68. The functional relevance of this ligand/receptor interaction was supported by the fact that blocking the Tim-3 pathway *in vitro* recovered IFNγ and TNFα - producing HBV-specific CD4 and CD8 T cells in more than 50% of CHB patients. Responses from patients with viraemia well-suppressed on antivirals maintained increased expression of Tim-3 and recovery of functional responses upon blockade of this co-inhibitory pathway. The development of strategies to manipulate this interaction could therefore contribute to restoring antiviral T cells responses and the long-term control of HBV infection.

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The role of transcription factor Nrf2 in T-cell development and function

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T cells are principal cells of the adaptive immune system involved in the immune responses to pathogens. Mature CD4 and CD8 T cells are generated following complex developmental and selection processes that takes place in the thymus. Mature T cells that populate the secondary lymphoid organs are competent in antigen recognition, antigen receptor signalling, clonal expansion, effector functions and in the generation of immunological memory. Evidence has shown that the intracellular redox equilibrium of the T-cell is imperative for optimum immune functioning. Alterations in redox balance can result in dysfunctional T-cell signalling and impact on the subsequent immune response elicited. The transcription factor Nrf2 maintains cellular redox homeostasis via upregulation of cytoprotective and antioxidant genes in response to oxidative or chemical stressors. Little is known of the role of Nrf2 in the regulation of T-cell development and mature T-cell function. Using T-cells derived from $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice, we demonstrate that loss of Nrf2 does not affect T-cell development in the thymus or the proportions of naive CD8 and CD4 T-cell populations in secondary lymphoid organs. Furthermore, Nrf2 does not influence naive or effector T-cell proliferation in response to stimulation with CD3 and CD28 antibodies. However, distinct cytokine profiles between $Nrf2^{+/+}$ and $Nrf2^{-/-}$ effector T-cells were observed. Taken together, this data suggests that Nrf2 may play a role in peripheral T-cell function but not T-cell development.

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Study of the activity of two isoforms of the transcription factor PAX5 in the terminal maturation of B lymphocytes

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PAX5 is a transcription factor essential for the B lymphocyte development. Multiple isoforms result from alternative splicing events and from transcription initiation at two promoters (PA and PB) associated with two alternative first exons. PAX5A is synthetized from the transcript of exons 1A and 2−10 from PA, PAX5B uses PB and exon 1B. These isoforms differ only in the sequence encoded by the first exon. PAX5A is expressed specifically in the LB and PAX5B expression appears similar but is still unclear.

PAX5A activates B cell specific genes and represses genes associated with the differentiation of other cell lineages. PAX5B, appears *in vitro* to prevent apoptosis and negatively regulates t the promoter of CD19 in B cell lines. We have shown that both PAX5A and PAX5B were able to activate transcription of the CD19 promoter in non-lymphoid cell lines. However, PAX5B is no longer able to perform this function in a B cell line, whereas the ability of PAX5A transactivation remains intact. We observed that these two isoforms show a differential regulation in activated cells: mPAX5A transcript is decreased following activation while mPAX5B is increased. The 3'regulatory region (3'RR) of the IgH locus, which is particularly active during the terminal maturation of plasma cells, is known to be a target of PAX5. We observed an increased binding of PAX5 to the 3'RR after B cell activation. These results suggest that the balance between PAX5 isoforms PAX5A and PAX5B regulates the function of PAX5 during terminal maturation of LB.

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The immunoglobulin heavy-chain locus 3′ regulatory region (hs3a, hs1,2, hs3b, hs4) is dispensable for VDJ assembly

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The immunoglobulin heavy chain locus undergoes numerous genomic rearrangments during B cell maturation (VDJ recombination, class switch recombination, somatic hypermutation). These differents events are regulated by several *cis*-regulatory elements. A 3′ regulatory region (3′RR) located downstream the IgH locus has been shown to contain four lymphoid-specific transcriptional enhancers: hs3a, hs1,2, hs3b and hs4. To understand the role of this 3′RR in B-cell development, the laboratory created a knock-out murine strain deficient for the whole 3′RR (3′RR deficient mice). Previous results shown that the complete 3′RR deletion dramatically affects class switch recombination and Ig secretion for all isotypes (Vincent-Fabert et al, Blood 2010 116: 1895–1898). We use this murine model to investigate the role of the 3′RR in V(D)J recombination. We firstly tested the 3′RR implication in the choice of a specific IgH allele in heterozygous mice. Results demonstrated, in $IgMa^{\Delta 3'RR}$/IgMb animals, the similar use of either 3′RR-deleted allele or wild-type allele for IgM synthesis in bone marrow. The diversity of rearrangement as well as the V, D and J usages were, in $CD25^+$ pre-B cells or mature splenocytes, not affected by the 3′RR deletion. Taken together, these results reveal no evident role for the 3′RR during V(D)J recombination process. Currently, studies are in progress to investigate the role of the 3′RR in somatic hypermutations.

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Melioidosis and correlates of protection

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Burkholderia pseudomallei is an opportunist pathogen found in South East Asia and Australasia that causes the fatal disease melioidosis in humans. *B. pseudomallei* is intrinsically resistant to many antibiotics and there is currently no licensed vaccine. Here we investigate the effect of three pre-infections by attenuated Burkholderia. The protective and attenuated mutant 2D2 was derived from a virulent *B. pseudomallei* strain and is a branch-chain amino acid auxotroph. *Burkholderia thailandensis* is a close relative to *B. pseudomallei* and is avirulent in humans. We have obtained a *B. thailandensis* strain (cap^+) that has a capsule similar to that of *B. pseudomallei* and a closely related strain (cap^-) that lacks any capsule. Using a two sequential challenge experiment, we show that the cap^+ strain provided comparable protection to the 2D2 vaccine and cap^- provided limited protection (bacterial colonisation and survival). We found that pre-challenge IgG levels correlated with bacterial burden post-challenge. This allows us to establish correlates-of-protection that would be required to indicate whether vaccinated humans would be protected from melioidosis by vaccination. Additionally, we find that a pre-infection with the non-pathogenic *B. thailandensis* cap^+ gave similar efficacy to the 2D2 vaccine, which is unlikely to be licensed due to safety issues.

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The unexpected T-cell recognition of an altered peptide ligand is driven by reversed thermodynamics and an alternative structural hotspot

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The molecular basis underlying T-cell recognition of MHC molecules presenting altered peptide ligands (APLs) is still not well established . A hierarchy of T-cell activation by MHC class I-restricted APLs has been defined using the T-cell receptor P14 specific for $H\text{-}2D^b$ in complex with the immunodominant LCMV peptide gp33 (KAVYNFATM). While substitution at peptide position 4 of the main TCR-interacting tyrosine residue to either phenylalanine (Y4F) or to serine (Y4S) abolished recognition by P14, the TCR unexpectedly recognized $H\text{-}2D^b$ in complex with the alanine-substituted semi-agonist Y4A, which displayed the most significant structural modification. The observed functional hierarchy could not be explained by the relative capacity of the peptides to stabilize $H\text{-}2D^b$. Analysis of the thermodynamic signatures revealed that while recognition of the full agonist $H\text{-}2D^b$/gp33 by P14 was enthalpy-driven, recognition of the semi-agonist $H\text{-}2D^b$/Y4A was primarily entropy-driven. Comparative crystal structure analyses revealed that the conformations of peptide residue p1K and of the key surrounding heavy chain residues E58 and R62 are different in $H\text{-}2D^b$/Y4A compared to the three other MHC molecules. The side chains of the two MHC residues move closer together in $H\text{-}2D^b$/Y4A, thereby possibly creating with p1K a new alternative hotspot for interaction with P14. Molecular modeling indicated that P14 favorably adapts to this alternative structural hotspot in $H\text{-}2D^b$/Y4A through a lateral hinge bend movement of its CDR1a. This results in increased interactions between P14 CDR1a and residues p1K, E58 and R62 in $H\text{-}2D^b$/Y4A that could account for the unexpected recognition of the semi-agonist complex.

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TH9 cells promote mast cell mediated allergic pathology in the lung *in vivo*

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IL-9 secreting (TH9) T cells represent a distinct T cell subset. However evidence for their functionality in disease is uncertain. We measure directly TH9 cells in patients and show that circulating TH9 cells are elevated in allergic compared with non atopic donors which correlates with increased IgE levels ($P < 0.05$ $r^2 = 0.50$). Using a murine model of house dust mite (HDM) induced allergic airways disease, allergen challenge leads to rapid TH9 differentiation and proliferation, with much faster kinetics than TH2 cell differentiation (6.2×10^3 versus 1.9×10^3 cells/ml 1 week post first HDM challenge, $P < 0.001$) resulting in recruitment and activation of mast cells. Adoptive transfer of TH9 cells results in enhanced pulmonary inflammation, eosinophilia (2.9×10^4 versus 1.8×10^4 siglecF$^+$ cells/ml) and intra epithelial mast cells numbers (27 versus per mm). Mast cells were activated as evidenced by increased serum mast cell protease levels (38 versus 22 ng/ml) and circulating IgE levels were also enhanced in mice receiving TH9 cells (0.97 versus 0.48 μg/ml). Conversely, inhibition of HDM induced TH9 differentiation results in significantly reduced airway hyperreactivity, collagen deposition, mast cell numbers and activation. Thus we define a functional phenotype for TH9 driven pathology *in vivo* that is different but complimentary to that elicited by TH2 cells. TH9 cells promote allergic responses, resulting in enhanced mast cell mediated pathology in the lungs.

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Proteinkinase D2: a critical diacylglycerol regulated threshold sensor for the T cell antigen receptor

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Protein Kinase D2 (PKD2) is a diacylglycerol and Protein Kinase C regulated serine/threonine kinase that is activated by triggeringof antigen receptors in T cells. An unbiased analysis of the serine/threonine phosphoproteome of TCR triggered PKD2 null CD8 T cells using high resolution mass spectrometry reveals that this single serine kinase controls a signal transduction network. Consequently, PKD2 has a key role in the transcriptional reprogramming of CD8 naive T cells that occurs following antigen receptor engagement. In particular, PKD2 controls the ability of CD8 T cells to respond to antigen and produce key proinflammatory cytokines notably Interferon gamma. Strikingly, Interferon gamma output in T cells is tightly linked to the level of PKD2 activity. This is relevant because the level of PKD2 activity in naive T cells is regulated in an analogue response where the stoichiometry of PKD2 activity is determined by the strength of the TCR ligand. PKD2 is thus a critical diacylglycerol regulated threshold sensor that translates TCR signaling strength to a functional outcome.

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Classical and non-classical class II genes in a non-mammalian vertebrate

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Previous work by our lab has shown the chicken major histocompatibility complex (MHC) to be smaller, simpler and re-arranged compared to mammals, with stable haplotypes leading to polymorphic gene loci that can co-evolve. For example, co-evolution of the dominantly-expressed class I molecule with TAP and tapasin can explain the strong associations of the chicken MHC with resistance or susceptibility to particular pathogens.

Following from this, we are investigating whether the presence of a dominantly expressed chicken class II molecule can also be explained by co-evolution with associated antigen processing genes, the non-classical chDM genes. To this end we characterised the previously poorly defined chDM region and investigated the expression, regulation, and interaction of the class II (B-L) and chDM genes.

We find that chickens are unusual in having two DMB genes (chDMB1, chDMB2) and an alternative first exon for the alpha-chain gene (chDMA). We identify putative promoters and further regulatory sites throughout the chDM region. We find classical (B-LA, B-LB1, B-LB2) and non-classical (chDMA, chDMB1, chDMB2) class II genes expressed in cell lines and tissues. B-LB2 and chDMB2 are dominant while B-LB1 and DMB1 are elevated only in particular tissues.

This work provides a detailed description of the genes and potential regulatory elements of the DM region in a non-mammalian vertebrate. In addition, and in contrast to previous assumptions that BLB1 and chDMB1 are barely expressed or probable psuedogenes, we show tissue specific elevated expression, leading us to investigate whether these genes may be co-regulated and have an unusual function.

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Inflammatory mediators involved in scorpion envenomation pathogenesis: characterization of immune response

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Clinical symptoms observed after scorpion stings have been mainly attributed to the pharmacological actions of the neurotoxins of venom on their targets. This binding produces abnormal neurotransmitter and inflammatory mediator release leading to severe pathological effects. This study describes evaluation of systemic inflammatory response after scorpion envenoming. Analysis of tissue damage and inflammatory response was carried out into mice envenomed with Androctonus australis hector (Aah) scorpion venom. Induced inflammatory process by Aah venom is characterized by tissue hyperleukocytosis, hemorrhage and inflammatory edema in most vital organs (lungs, heart, liver and kidneys). Lipid peroxidation products, including malondialdehyde (MDA), nitric oxide (NO) products, including nitrite, nitrate were found to be significantly elevated with a concomitant depletion of antioxidants in envenomed mice as compared to normal controls. In the blood sera, fast kinetic production of pro-inflammatory cytokines (IL1-β, IL-6, TNF-α) accompanied by hyper-gammaglobulinemia and activation of complement system were correlated with the severity of envenomation. The pro-inflammatory cytokines play an important role in the cell recruitment and the activation of mediators responsible for the later inflammatory response and the repair of tissue damages. The pathophysiological effects caused after envenomation may be mediated, in part, by cytokines and cytotoxic leukocyte-derived product release such as cationic proteins and possibly reactive oxygen/nitrogen species. Early treatment after scorpion stings with specific drugs that inhibit cytokine production, may have a potential beneficial effect to attenuate the observed clinical symptoms.

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Reversible senescence in human $CD4^+$ $CD45RA^+CD27^-$ memory T cells

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Persistent viral infections and inflammatory syndromes induce the accumulation of human T cells with characteristics of terminal differentiation or senescence. However the mechanism that regulates the end-stage differentiation of these cells is unclear. Human $CD4^+$ effector memory T cells ($CD27^-CD45RA^-$; EM) and also those that re-express CD45RA ($CD27^-CD45RA^+$; EMRA) have many characteristics of end-stage differentiation. These include the expression of surface KLRG-1 and CD57 reduced replicative capacity, decreased survival and high expression of nuclear gH2AX after T cell receptor (TCR) activation. A paradoxical observation was that although $CD4^+$ EMRA T cells exhibit defective telomerase activity after activation, they have significantly longer telomeres than central memory-like (CM; $CD27^+CD45RA^-$) and effector memory-like (EM; $CD27^-CD45RA^-$) $CD4^+$ T cells. This suggested that telomerase activity was actively inhibited in this population. Since pro-inflammatory cytokines such as TNF-a inhibited telomerase activity in T cells via a p38 mitogen- activated protein kinase (MAPK) pathway, we investigated the involvement of p38 signalling in $CD4^+$ EMRA T cells. We found that the expression of both total and phosphorylated p38 was highest in the EM and EMRA compared to other $CD4^+$ T cell subsets. Furthermore, the inhibition of p38 signalling, especially in $CD4^+$ EMRA T cells, significantly enhanced their telomerase activity and survival after TCR activation. Thus activation of the p38 MAPK pathway is directly involved in certain senescence characteristics of highly-differentiated $CD4^+$ T cells. In particular, $CD4^+$ EMRA T cells have features of telomere-independent senescence that are regulated by active cell signalling pathways that are reversible.

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An mTORC1/ARNT axis coordinates the glycolytic switch in effector CD_4 and CD8 T cells

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The mammalian target of rapamycin complex 1 (mTORC1) controls the differentiation of peripheral CD4 and CD8 T cells. The present study reports a key role for mTORC1 to control glucose metabolism and glycolysis in immune activated T cells. Importantly, mTORC1 signaling controls the expression of the Glut1 - glucose transporter by regulating the expression of Hif1a/ARNT transcription factor complex. Naïve T cells do not express the Hif1a/ARNT complex. However, the expression of this complex is induced in response to triggering of the T cell antigen receptor complex and is then sustained by cytokines notably IL-2 and the Th17 polarizing cytokines IL-1, TGF beta and IL-6. To examine the role of Hif1a/ARNT complexes in T cells we selectively deleted ARNT alleles in T cells. ARNT null CD4 T cells showed abnormal glucose metabolism, Th17 differentiation and failed to produce the cytokine IL-22. Immune activated ARNT- null CD8 T cells also failed to differentiate normally to effector CTLs. Immune activated Arnt null CD8 T cells thus fail to normally express Glut-1 and have low rates of glucose uptake and glycolysis. Strikingly, despite these glycolytic defects ARNT is not required for CD8 T cell proliferation or protein synthesis. However, immune activated CD8 T cells ARNT - null CTLs fail to downregulate the expression of the lymph node-homing receptors CD62L (L-selectin) and CCR7. They also fail to express the cytolytic effector molecule perforin. These data reveal an mTORC1/ARNT axis coordinates the glycolytic switch that is required for effector CD4 and CD8 T cell differentiation.

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Analysis of recombinant monoclonal antibodies from single B cells reveals early defects of B cell tolerance checkpoints in patients with Sjögren's syndrome

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Background: Sjögren's syndrome (SS) is an autoimmune disease characterized by high affinity circulating autoantibodies and characteristic B cell disturbances with a predominance of naïve and a reduction of memory B cells in the periphery. It is unknown at what stages of B cell differentiation tolerance checkpoints are defective in SS. Here we aimed to determine the frequency of self-reactive B cells in the circulating naïve compartment of SS patients.

Aims and Methods: Single IgD+ CD27− naïve (and CD27+ memory) B cells were FACS sorted from seven SS patients and RNA used to amplify Ig VH and VL genes which were then cloned and expressed as recombinant monoclonal antibodies displaying an identical specificity of the original B cells. B cells from healthy donors (HD) were used as control. Recombinant antibodies were tested towards different autoantigens to determine the frequency of autoreactive clones.

Results: A total of 80 individual recombinant antibodies were generated from naïve and memory B cells of SS patients. Analysis of the VH and VL gene usage showed no significant differences between SS and HD. Self-reactive B cell clones displaying ANA and ENA reactivity were expressed by 42% of peripheral naïve B cells in SS patients, significantly higher compared to HD (∼10%). Interestingly, most naïve self-reactive antibodies were polyreactive.

Conclusions: Here using a novel strategy to express recombinant antibodies from single B cells we demonstrated an elevated frequency of autoreactive naïve B cells in the circulation of SS patients. This evidence likely reflects early defects in B cell tolerance checkpoints.

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Validation of immunoCAP ISAC assay performance

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ISAC is a microarray technique using allergen components. Currently few laboratories provide this assay in UK. Currently most component resolved diagnostic prognostication is on the ImmunoCAP system. ISAC is a semi-quantitative method with multiple allergens on a single assay and has potential advantages.
Method: We analyzed clinic letters and identified a cohort of patients ($n = 50$) in whom allergen component specific IgEs (sIgE) were indicated. ISAC were performed on stored sera on which component sIgEs have been measured on Immunocap1000 previously. The results from both systems were compared with the clinical outcomes.
Results: ISAC shows good agreement with ImmunoCAP1000 results. In this cohort we have examined peanut, hazelnut and latex. The agreements were 100‰ in Ara h 1, Bet v 2 and Hev b 8; 95‰ in Ara h 3 and Bet v 1; 88‰ in Ara h 2; 85‰ in Ara h 8 and Hev b 6.
Conclusion: ISAC is less sensitive and its calibration characteristic is less robust than the ImmunoCAP1000 in measuring the component sIgEs. Correlation of component patterns with clinical diagnoses is good for both systems. The numerical value is not comparable between them. The ISAC profile does not currently include all major relevant components e.g. Ara h 9. High total IgE does not appear to non-specifically affect the component results. ISAC has potential advantages - cost effectiveness, efficient sample usage, shorter turn-around time for multiple testing and providing a cross-sensitization profile. ISAC should be interpreted with clinical details. The report should be coherent and practical.

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Induction of Treg and Th17 cells by pneumococcus in nasal-associated lymphoid tissues and their association with pneumococcal carriage in children and adults

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Background: Regulatory T (Treg) and Th17 cells play a vital role in mediating immunity to microbial infection. We show previously that pneumococcus induces Treg formation in human adenoids, part of the nasal-associated lymphoid tissue (NALT). In this study we aim to investigate the mechanisms by which pneumococcus induce Treg and Th17 cells and their effect on T and B cell immunity and their association with pneumococcal carriage in children and adults.
Methods: Mononuclear cells from adenotonsillar tissues are isolated from children and adults undergoing adenotonsilectomy. Cells are then stimulated by concentrated pneumococcal culture supernatant derived from a type II pneumococcus D39 followed by intercellular staining of Foxp3 and Interleukin 17 and other relevant cytokines. Antibody and cytokine production are assayed by ELISA to compare between the Treg-depleted cells and the non-depleted cells.
Results and conclusion: Both $Foxp3^+$ Treg and Th17 cells are induced by pneumococcal stimulation. Preliminary results suggest that there is a difference in the ratio of Foxp3+ Treg to IL17-producing cells between children and adults, which tends to be higher in the former in whom pneumococcal carriage is more common. Depletion of Treg cells significantly enhanced the antigen-specific antibody production. It is suggested that the development and balance of $Foxp3^+$ Treg and Th17 cells in the local mucosal immune tissues play an important part in modulating the specific immunity and pneumococcal carriage in humans.

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B cell derived interleukin-6 drives pathogenesis in experimental autoimmune encephalomyelitis and multiple sclerosis

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B cells have paradoxical roles in autoimmunity, exerting both pathogenic and protective effects. Pathogenesis may be antibody-independent as B cell depletion therapy (BCDT) leads to amelioration of disease irrespective of autoantibody ablation. However, the mechanisms of pathogenesis are poorly understood. Here we demonstrate that B cells contribute to pathogenesis of experimental autoimmune encephalomyelitis (EAE) primarily through secretion of interleukin (IL)-6. B cells from mice with EAE secreted elevated levels of IL-6 compared to B cells from naïve controls, and mice with a B cell-specific IL-6 deficiency showed a markedly less severe disease than mice with wild-type B cells. Moreover, BCDT ameliorated EAE only in mice with IL-6 sufficient B cells. This mechanism of pathogenesis also operated in multiple sclerosis (MS) because B cells from MS patients produced more IL-6 than B cells from healthy controls. This abnormality was normalized in B cells returning after Rituximab treatment, suggesting that BCDT improved disease progression at least partly by eliminating IL-6 producing B cells in MS patients. Taking these data together, we conclude that IL-6 secretion is a major mechanism of B cell-driven pathogenesis in T cell-mediated autoimmune disease such as EAE and MS.

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Characterization of THEMIS as a new member of the TCR signalosome

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Stimulation of the T cell antigen receptor (TCR) induces formation of phosphorylation-dependent signalling networks, ultimately leading to T cell proliferation and differentiation. Despite decades of research, the composition and dynamics of the TCR signalosome is still incompletely understood. Others and we have recently identified Thymocyte-expressed molecule involved in selection (THEMIS), as a novel regulator in thymocyte positive selection. The exact role of THEMIS in signalling, especially in peripheral T cells, has remained poorly characterized and controversial.

In the present study we show that THEMIS is a new member of the TCR-proximal signalosome. We are able to demonstrate that THEMIS acts as a positive regulator of TCR-induced IL-2 gene expression, via modulation of ERK and NFAT activity. Upon TCR triggering, THEMIS is rapidly tyrosine phosphorylated by the Src family kinase Lck. We find this phosphorylation dependent on the presence of the scaffold proteins Linker for activation of T cells (LAT) and SH2 domain-containing lymphocyte protein of 76 kDa (SLP-76). Phosphorylation of THEMIS coincides with its recruitment to LAT, via the adapter molecule growth factor receptor-bound protein 2 (GRB2). GRB2, in turn, is found to be constitutively bound to a highly conserved proline-rich region (PRR1) at the C-terminus of THEMIS. We can show that PRR1 is indispensable for GRB2 association, THEMIS Tyr-phosphorylation and function *in vitro* and for thymocyte development *in vivo*. Taken together, our study positions THEMIS as a regulator of proximal TCR signalling and further shows that the association of THEMIS with the LAT-signalosome is crucial for thymocyte development.

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Analysing the role of ERK MAPK signaling in tolerance and priming of antigen-specific $CD4^+$ T cells *in vitro*

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T cell recognition of Ag can result in priming or tolerance depending on the context in which Ag is recognized. Peripheral tolerance plays an important role in preventing T cells response to self or harmless antigens. One of the mechanisms which contributes to forming tolerance is anergy, which can be defined as defect in cellular proliferation and IL-2 production. Our lab has reported that there are significant differences in the amplitude and cellular localization of phosphorylated ERK (pERK) signals when naïve and *in vitro*-primed and tolerized T cells respond to Ag. GTPase Rap1 has been reported to inhibit the generation of pERK signals and to accumulate in tolerant cells. Consistent with this, our lab has demonstrated that Rap1 exhibits an inverse pattern of expression to pERK in individual Ag-specific primed and tolerized T cells. We have extended these studies to investigate whether Rap1plays a role in determining commitment to anergy and priming during induction and maintenance phases. Specifically, we have used an adenoviral gene transfer approach to examine the impact of Rap1 signaling on ERK activation, cellular proliferation and clonal expansion of Ag- specific $CD4^+$ T cells.

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A novel role for NFkB signalling in T cell homeostasis

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T cell development in the thymus and peripheral homeostasis depends critically on TCR and IL7R signalling. NFkB signalling has been implicated as an important regulator in cell survival and fate decisions and is activated downstream of TCR triggering. Using transgenic and gene knock-in mouse systems that allow conditional deletion of IKK1 and/or IKK2 in T cells, we evaluated the role of NFkB signalling in T cell development and peripheral homeostasis. Loss of both IKK1 and IKK2 in thymocytes revealed that maturation of single positive, but not double positive thymocytes was strictly dependent on NF-kB signalling. IKK1 and IKK2 acted redundantly in this maturation since thymic development in single IKK1 or IKK2 deficient mice was normal. In contrast analysis of peripheral T cell homeostasis revealed differential requirements for IKK1 and IKK2 expression. While IKK1 deficient T cells appear normal, survival and homeostatic proliferation of naïve T cells is dependent on IKK2 expression in a cell autonomous manner. Our results reveal qualitatively distinct requirements for NF-kB signalling during T cell development and homeostasis.

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The crystal structure of latent and immunodominant Epstein–Barr virus-derived T cell epitope

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Epstein-Barr virus (EBV) is a flourishing microparasite presently infecting over 90% of the human population. The virus is also a global disease burden and associated with 18 different malignancies. The latent EBV protein LMP2 is a key target of the cellular immune system since it encodes a number HLA-A*0201 (A2) restricted epitopes. Arguably the most potent LMP2 target is the CLGGLLTMV (CLG) peptide. CLG is a promising therapeutic and prophylactic target since it is expressed on the surface of most EBV^+ malignancies and exhibits complete sequence conservation across all known virus strains. How T cells engage this important epitope is unknown. Here, we have solved the structure of the CLG-A2 molecule and have identified key areas of T cell contact. Understanding the molecular basis of this engagement may aid in optimizing T cell recognition as well as the design of intelligent CLG mimics compounds and super-agonists for use in the clinic.

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Small intestinal CD103$^+$ DC are a heterogeneous population with distinct functions

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CD103 expressing dendritic cells (DC) play a crucial role in inducing tolerance in the gut. Two subpopulations of CD103$^+$ DC can be identified based on the expression of CD11b and CD8a molecules, but their respective functions in the small intestinal lamina propria (LP) remain unclear.

Both subsets express CCR7 and most TLR, as well as the retinoic acid generating enzyme ALDH, but CD11b$^+$ cells uniquely express CD172a and produce IL10 *in vivo*. CD11b$^+$ and CD8a$^+$ dendritic cells are both capable of antigen presentation when assessed *in vitro*, but there are differences in their overall potency and ability to prime CD4$^+$ or CD8$^+$ T cells.

Those results indicate that CD103$^+$ LP DC are a heterogeneous population whose two main subsets may have overlapping, as well as distinct functions. Although both are capable of acquiring intestinal antigen and migrating to the draining mesenteric lymph node to imprint gut homing properties on naïve T cells, there seem to be subtle differences in the nature of the response generated by each subset. It will be important to confirm these ideas directly and to explore how the subsets change in inflammatory conditions.

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SIRPα expression identifies two distinct populations of intestinal CD103$^+$ DC and contributes to DC homeostasis in the lamina propria

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The intestinal immune system is challenged continually by a variety of antigens, including commensal bacteria, food proteins and invasive pathogens. It is essential to discriminate between the different types of antigens so that either protective immunity or tolerance is induced appropriately. Dendritic cells (DC) that express CD103 are considered the archetypal intestinal DC involved in induction of tolerance, but the DC involved in active immunity are less well characterised. Here we show that intestinal CD103$^+$ DC are a heterogeneous population which can be subdivided based on the expression of signal inhibitory regulatory protein α (SIRPα), a receptor that binds the ubiquitously expressed CD47 and has several immunomodulatory roles. SIRPα is confined to the CD11b$^+$ subset of CD103$^+$ DC and the numbers of these DC are reduced in the intestinal lamina propria (LP) and MLN of mutant mice with impaired SIRPα signalling. Preliminary studies indicate that the lack of DC is not due to a defect in pre-DC generation or development, but may be due to poor survival of mature DC. Thus SIRPα may play a selective role in the homeostasis of a major subset of intestinal CD103$^+$ DC and future studies aim to explore further the consequences of defective SIRPα signaling for intestinal immune responses.

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Differential protein immunogenicity and allergenicity: role of glycosylation

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Human native milk lactoferrin (NLF) and recombinant forms of lactoferrin (rLF) are available with identical amino acid sequences, but different glycosylation patterns. Native LF has a complex glycoprofile including sialic acid and Lewis (Le) x structures, whereas both rLF forms are far simpler and rich in mannose residues. We have characterised antibody responses induced in BALB/c strain mice by NLF and rLF produced in either rice or *Aspergillus*.

Mice received various % w/v of NLF, rLF, ovalabumin or peanut alone or in combination by i.p. on days 0, 7. For adjuvant experiments with Le x, mice received an additional immunisation on day 14. Sera were analysed for protein specific IgG and IgE by enzyme-linked immunosorbant assay (ELISA) and homologous passive cutaneous anaphylaxis assay (PCA), respectively.

Immunisation with NLF stimulated vigorous IgG and IgE antibody responses, whereas both forms of rLF were 40-fold less immunogenic and 200-fold less allergenic, irrespective of endotoxin or iron content and the glycans did not contribute to epitope formation.

Endogenous expression of Le^x on NLF did not completely account for the more vigorous IgE responses provoked by this form of LF. Furthermore, co-administration of rLF and NLF inhibited the IgE response provoked by NLF and up-regulated IgG2a production, but was without effect on unrelated allergens.

Taken together, these data demonstrate that rLF impacts on the induction phase to selectively inhibit anti-LF IgE antibody responses and that differential glycosylation may impact on antigen uptake, processing and/or presentation influencing the Th1/Th2 balance.

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The regulatory role of CX3CR1 in gut macrophage function

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Inflammatory bowel diseases (IBD) have become a very important disease in industrialized countries. Macrophages (mf) in the intestinal lamina propria (LP) are the first point of contact between the immune system and the local bacteria but normally these mf are hyporesponsive to inflammatory stimuli. Fractalkine (FKN − CX3CL1) is produced by intestinal epithelial cells and has been shown to have a variety of modulatory effects on mf. Recent results have suggested that mice lacking CX3CR1 expression have a defect in orally induced tolerance due to failure of resident intestinal mf to drive regulatory T cell expansion via IL10. As resident intestinal mf express very high levels of CX3CR1, whereas pro-inflammatory mf that appear in experimental colitis express only intermediate levels, these findings suggest that the FKN-CX3CR1 axis may play an important role in regulating intestinal mf function. Here we show that resting CX3CR1 KO mice have normal numbers and subsets of colonic mf and recruit inflammatory class II MHC^{hi} CX3CR1^{int} Ly6C^{hi} mf during DSS colitis. Furthermore mf from CX3CR1 KO mice respond normally to TLR stimulation and exogenous FKN has no consistent effects on wild type mf function. There are also no clear differences in the outcome of DSS colitis in CX3CR1 KO mice, or in the ability of KO mice to be primed by feeding soluble antigen + cholera toxin as an adjuvant. Together these findings suggest that the CX3CR1-FKN axis is not essential for intestinal mf homeostasis and more work may be required to establish its precise role.

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Alterations in killer immunoglobulin-like receptors (KIR) expression and functional activities of CD56+CD3+ cells in lung cancer

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CD56+ T cells were studied in samples of peripheral blood from small cell lung cancer (SCLC) (30 samples) and non-small cell lung cancer (NSCLC) (67 samples) patients compared to healthy controls (69 samples). The Killer immunoglobulin-like receptors [CD158a, CD158b and CD158e (inhibitory receptors)] and their ligands have been analyzed using flow cytometry. In addition, the CD56 T cells functional activity in healthy subjects and lung cancer patients has been identified by examining IFN-γ production, CD25 and CD69 expression. Our results show that the relative numbers of CD56+CD3 cells were increased in NSCLC ($P = 0.001$) and SCLC ($P = 0.002$). The expression of the killer-immunoglobulin-like receptor CD158a was significantly lower on CD56+CD3+ cells in SCLC than controls, and also in early stage compared to late stage non-small cell lung cancer patients. Mean levels of CD158e were higher in NSCLC patients than controls. CD158e levels on CD56+CD3+ cells were increased in the presence of its ligand HLA-Bw4 compared to controls. The ability of CD56+CD3+ cells to respond to activation by upregulating CD25 or producing interferon-γ were both significantly impaired. Also the percentage of CD69 was reduced insignificantly in NSCLC patients compared with the healthy control. Although the precise role of CD56+CD3+ cells is not clear, they appear to be functionally impaired in lung cancer, which may have implications for a reduction of direct or indirect anti-tumour responses.

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CD99 is epigenetically down-regulated in Ramos cells, representative of early centroblastsv

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The Ramos cell line, derived from Burkitt's lymphoma with centroblastic phenotype, has been chosen as a good model for studying on SHM, because of its constitutive mutation on the V regions of the immunoglobulin (Ig) genes. This cell line also shows a wide range of AID expression. In previous study, CD99 positive cells were scattered throughout the lymph nodes, but most of cells in germinal center had low expression of CD99 in immunohistochemical staining. To delineate maturation stages of B cells in the GC with an expression pattern of CD99, which has been reported to be down-regulated on the B cell in GC, we sorted and sub-cloned the Ramos cells according to the expression pattern of CD99 and investigated the events related to B cell maturation, such as IgM-induced apoptosis, Fas and AID expression in each clone. We observed that CD99+ clones have earlier centroblastic phenotypes than CD99− ones through experiments between CD99+ and CD99− clones, such as IgM-induced apoptosis and Fas expression. Moreover, CD99+ clones show high AID expression level, also have IgM- population, represent of SHM. $CD99^+$ clones become $CD99^-$ cells spontaneously however, the reverse event had not happened in any cases.

Recently, we have observed conversion of CD99− to CD99+ by 5-AZA deoxycytidine, demethylating agent. We will investigate the epigenetic regulation mechanism of CD99.

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Detection of CMV-specific T cells in transplant patients using MHC Dextramers

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Cytomegalovirus (CMV) infects and establishes persistent lifelong infections in 50−85% of adults. Reactivation of the virus is a frequent complication of immunosuppression e.g. following transplantation, and can significantly contribute to morbidity and mortality in such patients. CMV-specific T cells in the blood of immune-compromised patients is a good marker for protection against CMV disease. Sensitive and reliable monitoring of CMV-specific immune responses in transplant patients and other immune-compromised individuals could be used to predict which patients are at risk of developing CMV disease and thereby help guide the immunosuppressive and anti-viral treatment in such patients.

MHC Dextramers are a new generation of MHC multimer reagents that are used in flow cytometry to detect antigen-specific T cells in the blood. Dextramers carry a higher number of pMHC complexes and fluorochromes than the conventional MHC multimers. The higher number of pMHC complexes increases the Dextramer's avidity for the specific T-cells and the higher number of fluorochromes enhances staining intensity. As a result, Dextramers have higher sensitivity, staining intensity and signal-to-noise ratio than conventional MHC multimers such as Tetramers and Pentamers and thus provide a more reliable means for identification of true positive antigen-specific T cells.

We show how MHC Dextramers displaying peptides derived from CMV can be used for identification and accurate enumeration of CMV-specific $CD8^+$ T cells in the blood of stem cell transplant patients. The level of CMV Dextramer-positive cells may be a good indicator of patient immune status.

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MHC multimer assays: optimized conditions allowing enumeration of low-affinity, antigen specific CD4+ and CD8+ T cells by flow cytometry

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Reliable monitoring of cellular immune responses is important in vaccine and immunotherapeutic development. Flow analysis using conventional MHC multimers like Tetramers and Pentamers has made a great impact on this field, enabling enumeration and phenotypic characterization of antigen-specific T cells. However, it is often difficult to obtain well-separated, distinct negative and positive cell populations when attempting to detect T cells of low affinity for the MHC multimer.

Use of MHC Dextramers can overcome these problems, and provide reliable detection of low-affinity CD8+, CD4+ and iNKT cells. MHC Dextramers are an improved form of MHC multimers that improves staining intensity, resolution and signal-to-noise dramatically under these circumstances.

Cancer vaccines typically stimulate T cells with low affinity for the cognate pMHC class I complex. We show that MHC Dextramers, in contrast to Tetramers, can efficiently stain low-affinity T cells. Thus, using Dextramers, staining is achieved for affinities as low as 500 μM, and effective separation of the negative and positive cell populations is achieved for affinities as low as 250 μM.

pMHC class II complexes typically have low affinity for their cognate TCRs, making it difficult to enumerate CD4+ cells involved in e.g. autoimmune or inflammatory disease using conventional MHC multimers. We show that class II Dextramers allow detection of CD4+ T cells that cannot be detected using tetramers.

Finally, CD1d complexes (MHC-like complexes comprising glycolipids rather than peptides) often have low affinity for the TCR of iNKT cells. The use of CD1 Dextramers may be used to enumerate these cells.

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A phase I study to assess safety and immunogenicity of novel schedules for vaccination with candidate malaria vaccines ChAd63 ME-TRAP and MVA ME-TRAP

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Eight week prime-boost regimes using ChAd63, a simian adenovirus, and MVA, a recombinant Modified Vaccinia virus Ankara, with an ME-TRAP insert, have previously shown to be safe and induce sterile immunity in 21% of *Plasmodium* challenged volunteers and a delay to patency of malaria infection in 36%. Safety, immunogenicity and efficacy have only been assessed using a two vaccination prime-boost strategy with an 8 week interval. It is proposed that greater efficacy may be achieved if novel vaccination schedules are used.

In this phase I clinical trial, 42 healthy adult volunteers receive ChAd63 ME-TRAP (A) 5×10^{10} vp and MVA ME-TRAP (M) 2×10^{8} pfu, according to seven different vaccination schedules. The vaccinations are given intramuscularly and are of pre-established doses. The schedules all use a four vaccination regime of AAAM, AAMM, AMMM or AMAM, with varied time intervals of 4 or 8 weeks.

The primary objective is to assess the safety profile of the seven vaccination schedules, with the secondary objective to assess immunogenicity. The primary immunological readout is the *ex-vivo* IFN-γ ELISpot assay performed on fresh PBMCs, which are isolated from whole blood and stimulated with overlapping pools of T996 and 3D7 peptides, and a complete pool of ME peptides. These assays are performed at selected time points pre- and post-vaccination and will be used to compare the immunogenicity of the different schedules. Preliminary results have seen a good safety profile for all volunteers vaccinated to date.

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Characterisation of T cells induced by candidate prophylactic vaccines encoding a HIV-1 conserved immunogen in phase I/IIa trial HIVCOREo02

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To overcome HIV-1 diversity, we have designed a novel T cell immunogen, HIVconsv, a chimaeric protein encoding the 14 most conserved regions of the HIV-1 proteome from four major HIV-1 clades A, B, C and D.

This immunogen is vectored by plasmid DNA pSG2.HIVconsv (D), Chimpanzee Adenovirus Serotype 63 ChAdV63.HIVconsv (c/C) and Modified vaccinia virus Ankara MVA. HIVconsv (M). We are currently investigating vaccine-induced responses in healthy adults in a phase I/IIa clinical trial in Oxford, four stages will be used to assess 3 prime-boost regimes. Stage 1: c - Low dose ChAdV63 $n = 2$, Stage 2: CM $n = 8$, Stage 3: DDDCM $n = 8$ and Stage 4: DDDMC $n = 8$. In addition two placebo controls are included at Stages 2–4.

To date, volunteer PBMC from stages 1 and 2 are being assessed for frequencies of vaccine-induced T cells using the *ex vivo* IFN-γ ELISpot. Initial results for stage 1 showed that ChAdV63.HIVconsv was safe, well tolerated and immunogenic in all volunteers. Stage 2 group data showed peak responses to ChAdV63.HIVconsv prime at week 4 median of 630 SFU/10^6 PBMC (range 150–1470 SFU/10^6 PBMC) and this was boosted by MVA.HIVconsv at week 9 to a median 5150 SFU/10^6 PBMC (range 1475–16 495 SFU/10^6 PBMC).

At key timepoints, the CD8+ T cell capacity to inhibit viral replication within autologous CD4+ T cells will be examined in a Viral Suppression Assay. Data so far suggests that background suppression levels in HIV-1 negative individuals is low and that high levels of suppression can be detected following HIVconsv vaccination.

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Evaluation of immunological mechanisms induced by *Mycobacterium avium* exposure and the effect upon pre-existing BCG immunity

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BCG, the current vaccine against *M. tuberculosis* (*Mtb*), confers protection against disseminated disease in childhood, but the protection against pulmonary disease varies from 0% to 80%.It is becoming more apparent that interaction with non-tuberculosis mycobacteria (NTM) has an effect on the level of protection conferred by BCG vaccination. We hope that evaluation of immunological mechanisms induced by NTM will give us insight into the effect NTM exposure may have on immunity induced by current and candidate TB vaccines.

A model of *M. avium* (MA) exposure was designed to profile and compare immune responses induced by MA with those induced by BCG. The effect of MA exposure on pre-existing BCG immunity was modelled in C57bl/6 mice vaccinated with BCG then exposed to repeated doses of heat-killed MA. Mice were subsequently challenged with aerosolized *Mtb* and immunological parameters were assayed using flow cytometry and FlowCytomix cytokine assays.

There was no abrogation of Th1 responses in mice receiving MA after BCG vaccination. Upon infection with Mtb MA exposed mice had greater frequencies of IFNγ^+ CD4$^+$ and IL-17 + CD4$^+$ cells in the lung than mice receiving BCG vaccine alone. IFNγ+CD8$^+$ frequencies in lungs of mice exposed to MA were reduced.

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$\gamma\delta$ cells with adaptive-like characteristics are generated by thymic ligand-independent signalling

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$\gamma\delta$ T cells are prominent early producers of Interleukin-17 (IL-17) and Interferon-γ (IFN-γ). Their rapid response is a key inducer of downstream immunity; hence they have great potential to regulate host protection and the development of immunopathologies. Consequently, there is growing interest in the developmental origins of these $\gamma\delta$ T cell subsets. Previously we determined that most peripheral IL-17-producing $\gamma\delta$ cells are contained within a CD27$^{(-)}$ compartment, while IFN-γ-producing cells mostly express CD27, and that both populations are also present in the thymus. Current evidence suggests that, at least to some extent, $\gamma\delta$ T cells are pre-programmed during thymic development. It is believed that T cell receptor (TCR)-agonists favour the development of the IFN-γ producing subset, whereas IL-17 producing $\gamma\delta$ T cells develop *via* a default, antigen naïve pathway. This present study however, demonstrates that *in vitro*, $\gamma\delta$ T cells expressing a TCR lacking any extracellular immunoglobulin-like domains, and hence cannot engage ligand, commit towards a CD27$^{(+)}$ $\gamma\delta$ T cell fate and do not develop into IL-17 producing $\gamma\delta^{27-}$ T cells. Furthermore, these cells have a predominantly CD24$^{(-)}$CD122$^{(-)}$ phenotype, which is also readily identifiable in both the thymus and lymph nodes of wild-type mice. Significantly, CD24$^{(-)}$CD122$^{(-)}$ $\gamma\delta^{27+}$comprise the major IFN-γ producing $\gamma\delta$ T cell subset. This data suggests that the absence of TCR $\gamma\delta$/ligand interactions during $\gamma\delta$ T cell development promotes the generation of IFN-γ-committed $\gamma\delta^{27+}$cells that display 'adaptive-like' characteristics.

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Immunization with Newcastle disease virus capsids displaying the EV71 VP1 fragment stimulated antibody responses in hamster

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EV71 causes severe neurological diseases resulting in high infection incidences and mortality in young children worldwide. Development of an effective vaccine towards EV71 infection is hampered by the lack of appropriate animal models for efficacy testing of candidate vaccines. We have successfully tested the immunogenicity and protectiveness of a candidate vaccine containing a recombinant Newcastle disease virus capsids that display an EV71 VP1 fragment (NPt-VP1$_{1-100}$) protein in a mouse model of EV71 infection (Ch'ng et al., 2011). A drawback of this system is its limited window of EV71 susceptibility period, 2 weeks, leading to restricted options in the evaluation of optimal dose regimens. To address this issue, we have assessed the NPt-VP1$_{1-100}$ candidate vaccine in a hamster system, which offers a 4-week susceptibility period to EV71 infection. Results obtained showed that the NPt-VP1$_{1-100}$ candidate vaccine stimulated excellent humoral immune response in the hamsters. Despite the high level of antibody production, they failed to neutralize EV71 viruses. Following EV71 viral challenge studies, no statistically significant difference was observed in terms of survival and recovery in the NPt-VP1$_{1-100}$-immunized versus the control group. Even though, studies towards improving the construct are being attempted in our laboratory, findings from this study has contributed towards a better understanding of the NPt-VP11-100 recombinant protein as a candidate vaccine in an alternative animal system.

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Regulation of Ig class switching and plasma cell differentiation by IRF4 and NFκB1

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The transcription factor IRF4 is essential for immunoglobulin class switch recombination (CSR) and plasma cell differentiation. Whilst high levels of IRF4 induce terminal differentiation to plasma cells, low-level expression of IRF4 has been shown to be associated with CSR. To study the role of differential expression of IRF4 for CSR, we immunised quasi monoclonal (QM) mice, which have a high frequency of B cells specific for the model antigen 4-hydroxy-3-nitrophenylacetyl (NP), with NP-Ficoll. This induces B cell activation with CSR, followed by parallel differentiation of both plasma cells and T-independent germinal centre B cells. We show that low-level expression of IRF4 is induced within hours of B cell activation. This is associated with CSR, and precedes germinal centre or plasmablast development. NFκB1 has been shown to regulate IRF4; therefore to understand the regulation of differential expression of IRF4, NFκB1-deficient QM mice were produced. When QMxNFκB1^{KO} mice were immunised with NP-Ficoll, early low-level IRF4 expression was normal. However, 3 days after immunisation B blasts failed to differentiate into IRF4^{high} plasmablasts or IRF4^{low} germinal centre type cells. QMNFκB1^{KO} B cells transferred into wild type hosts were capable of producing small germinal centres but plasma cell numbers and antibody titres were drastically reduced. This shows a B cell intrinsic role of NFκB1 for the induction of high levels of IRF4. In summary, different signals regulate early and late expression of IRF4. Low-level IRF4 expression and CSR are independent of NFκB1, while NFκB1 is important for high-level IRF4 expression leading to plasma cell differentiation.

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Intestinal CD8α^+ dendritic cells constitutively migrate from the intestinal lamina propria to the mesenteric lymph node

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Dendritic cells (DCs) are specialised antigen presenting cells that continually migrate from the periphery to the mesenteric lymph nodes (MLN) where they prime naïve T cells. Intestinal DCs comprise at least two CD103-expressing subsets: CD11b$^+$ and CD8α^+ DCs. While CD11b$^+$ DCs are the major subset of lamina propria (LP) DCs, CD8α^+ DCs are more numerous in lymphoid tissues including the Peyer's Patches (PP) and isolated lymphoid follicles (ILFs). It is currently unknown whether CD8α^+ DCs constitutively migrate from the intestine and contribute to T cell priming in the MLN.

Thoracic duct cannulation, preceded by mesenteric lymphadenectomy, allows the isolation of all migratory intestinal lymph DCs (LDCs), without altering their phenotype. Our analysis of LDCs reveals two functionally distinct populations of CD103$^+$ DCs, expressing either CD11b or CD8α. In addition, both CD11b$^+$ and CD8α^+ DCs can be isolated from the LP. In order to identify the anatomical origin of the CD8α^+ LDCs, we used RORγt$^{-/-}$ mice, which lack all secondary lymphoid tissues including PPs and ILFs. Both the intestinal lymph and LP of the RORγt $^{-/-}$ animals contained the same proportions of CD11b$^+$ and CD8α^+ DCs as in wild type mice. These data unequivocally demonstrate that CD8α^+ DCs from the LP migrate constitutively to the MLN. Therefore, this population of migratory CD8α^+ DCs represents an important and previously unexplored therapeutic target for the treatment of inflammatory bowel diseases or the development of oral vaccines.

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Functional analysis of T cells lacking germline-encoded complementarity-determining regions

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The T cell receptor (TCR) recognises short antigenic peptides in the context of major histocompatibility complex (MHC) molecules. Many structural and functional studies suggest that the germline-encoded complementarity-determining regions (CDR) of the TCR are inherently biased towards recognition of MHC proteins. We have generated transgenic mice where all three β-chain CDRs were exchanged with short glycine and alanine-rich linkers which will have lost any inherent bias for MHC. In these mice, CD4$^+$ and CD8$^+$ T cells were selected to the periphery, although the selection efficiency and the size of the resulting CD4$^+$ and CD8$^+$ T cell compartments were reduced. These T cells were functional and responded to allogeneic stimulation *in vitro* by proliferating and producing IFN-γ. Moreover, transgenic mice lacking the hypervariable CDR3 region only, which functions to contact the MHC-bound peptide, were immunised with hen egg lysozyme (HEL). Immunised mice generated antigen-specific T cells which responded to *in vitro* HEL challenge by producing IFN-γ, demonstrating that these cells are able to recognise foreign antigen in a self MHC-restricted manner. The existence of functional T cells in mice lacking all three CDRs implies that these loops are not essential in T cell development and function. Therefore, we propose that the requirements for the selection of a functional repertoire are not entirely TCR-intrinsic and might be less strict than previously thought. In this context, the germline-encoded CDRs might play a role in regulating the diversification of the T cell repertoire and the size of the peripheral T cell pool.

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The roles of IL-25 and IL-33 in human CD4$^+$ T cell differentiation

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CD4$^+$ T cells are critical for effective immune responses, however environmental and genetic factors can lead to their contribution to disease. Asthma is a prime example, whereby the CD4$^+$ Th2 cell subset and associated cytokines are central to disease exacerbation. More recently, IL-25 and IL-33 have been demonstrated to enhance Th2 mediated responses and IL33 sequence variations were associated with increased blood eosinophil numbers and atopic asthma. Although both molecules are involved in Th2 responses, their mechanisms of action and cellular targets are not fully elucidated. This project therefore aims to investigate the role of IL-25 and IL-33 on human CD4$^+$ T cell differentiation. Using *in vitro* CD4$^+$ T cell differentiation and microarray analysis, we found both the IL-25 receptor (IL17RB) and the IL33 receptor (T1/ST2) to be selectively expressed by human Th2 cells. Verification by real-time PCR, western blotting and flow cytometry confirmed these findings. Interestingly, results demonstrated both the membrane bound and soluble decoy form of T1/ST2 to be Th2-specific. Treatment of naive CD4$^+$ T cells with IL-25 did not affect Th2 differentiation, however increased Th2 cytokine production from committed Th2 cells. In contrast, IL-33 enhanced Th2 cytokine expression at early stages of differentiation, suggesting their discrete roles in Th2 cell development.

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Developing an *in vitro* correlate for the effects of vaccine adjuvants on human CD4 T helper cell differentiation

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The differentiation of naïve T cells to distinct effector populations is central to the development of effective immune response.

We developed an in *vitro* assay to study CD4 naïve T cell commitment to effector cell differentiation with the purpose of investigating the influence of different adjuvants. We analysed compounds known to have distinctive Th1/Th2 biasing influences and as well a novel compound that activates invariant NKT cells (Compound-44). Compound-44 was compared against the effector responses seen in the presence of intracellular (R848) and extracellular (LPS) toll like ligands, iNKT agonist (a-GalCer) and Alum.

We studied the naïve T cell differentiation at day 10, in healthy controls following co-culture of whole blood with index adjuvants for 24 h and transfer of supernatants to CD3/CD28 bead activated naïve CD4 T cells. The effector responses were characterized by cytokine arrays, FACS and qPCR.

In the absence of adjuvant the default differentiation pathway was biased to Type 1 helper cells with a Th1:Th2 ratio of 10:1 and a 20:1 Th1:Th17 ratio. In cultures with R848/LPS the Th1:Th2 increased to 16:1 whilst the Th1:Th17 was 12:1. In the presence of the iNKT agonist a 3:1 Th1/ Th2 was observed with a 9:1 Th1/Th17 response. Alum generated a 1:3 Th1/Th2 and 1:2 Th1/Th17 response. Finally, compound-44 gave a distinctive pattern compared to a-GalCer with a 6:1 Th1/Th2 and a 7:1 Th1/Th17 response.

In vitro correlates of novel adjuvants may provide a useful adjunct to vaccine design strategies for distinct pathogen targets requiring bespoke effector responses.

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Immune system of rabbit does from different origin subjected to heat stress. preliminary results

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Stress is generally considered an important factor affecting the productivity of farm animals, as it is able to suppress the immune system and may lead to an increase in the occurrence of diseases in the presence of pathogens. In fact, it has been described that chronic heat stress can affect negatively the immune response in several production animal species, since they are genetically different and they show different ability to deal successfully with environmental challenges. Therefore, rabbits from different genetic lines subjected to heat stress also develop different immune system responses.

The present study aimed to determine and compare the ability of rabbit does from two different genetic lines selected by different productive criteria (one selected for productive longevity (LP) and one for litter size at weaning (V, using two generations of the same line: V16, V36), to deal with heat stress in terms of their abovementioned selection criteria.

The results pointed out that animals from the LP line showed a higher number of total lymphocytes (in blood). Furthermore, while the animals from line LP were able to modulate their immune response based on the total numbers of lymphocytes throughout the gestation-lactation cycle, animals from lines V16 and V36 did not show such ability to adapt to different situations, since the total cell number remained constant or decreased, reaching very low values in the second parturition. These results may suggest that, regarding immune response to thermal challenges, selecting by prolificacy criteria may have a negative impact on breeding rabbits.

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Notch controls CD8$^+$ memory T cell responses at three levels

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A fundamental property of the adaptive immune system is the generation of memory, which ensures superior defense against pathogens previously encountered. CD8$^+$ T cells protect against pathogenic viruses by killing virally infected cells. CD8$^+$ T cells initially exist in lymphoid organs as naive cells, which proliferate and differentiate into cytotoxic effector cells upon activation by antigen. After clearance of the infection, the majority of these effector cells is removed by apoptosis. A small proportion persists, however, and develops into memory cells, which are qualitatively different from naive T cells. The mechanisms and signals that determine the selective survival of memory precursors cells and their differentiation into functional memory cells are not clear. We now show that these processes are controlled by the Notch pathway. CD8$^+$ memory T cell responses to infection with Influenza virus are severely compromised in mice lacking expression of Notch1 and 2 receptors in CD8$^+$ T cells. Primary responses are largely normal in these mice, but are followed by precipitous disappearance of viral antigen-specific cells from blood and secondary lymphoid organs, resulting in an atrophied pool of memory precursor cells. The cells that do persist fail to expand upon recall infection and lack effector functions. We conclude that Notch governs memory responses to Influenza by controlling: (i) the number of cells entering/persisting in the memory pool after contraction, (ii) the ability of memory CD8$^+$ T cell to expand and (iii) memory CD8$^+$ T cell function.

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Transcriptome profiling of human Th9 cells

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Th9 cells are a recently described subset of T-effector cells that have the ability to express interleukin-9 (IL-9) and IL-10. Experimental data derived from mouse models suggests that Th9 cells can develop in the presence of the Th2 cytokine IL-4 and transforming growth factor b (TGFb). Here we show that human naïve CD4+ T-cells cultured with IL-4 and TGFb develop into cells Th9 cells capable of expressing IL-9 in the absence of Th1 (IFNg), Th2 (IL-4, IL-5, IL-13) or Th17 (IL-17) cytokines. In contrast to the mouse Th9 cells the human IL-9 positive cells do not co-express IL-10. Several other features of human Th9 cells also appear to differ from their murine counterparts. The transcription factors PU.1 and IRF4 have both been implicated in mouse Th9 cell differentiation, we observed no expression of PU.1 by human Th9 cells. IRF4 was widely expressed in all T-helper cell lineages with no evidence for selective expression in the Th9 subset. In common with mouse Th9 cells we observe high-level expression of the IL-25 receptor IL-17RB. Furthermore, to gain insight into factors that may be selectively expressed in human Th9 cells we have performed comparative microarray based transcriptome profiling.

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Characterisation of T-cell responses against human herpesvirus 6B in healthy subjects

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Human herpesvirus 6 (HHV-6) infects the majority of individuals in childhood, followed by a lifelong asymptomatic infection. Symptomatic disease is often observed in immune-compromised individuals, such as T cell-deficient transplant recipients. To date, HHV-6-specific T cell responses are ill-defined but, based on strong immune responses to other human herpesviruses, we hypothesised that HHV-6 will prove to be a significant immunogen for T cells. Thus, the aim of this study was to determine the base level of T cell responses against HHV-6 in healthy donors. We chose to screen for T cell responses against HHV-6B, the most common subtype of HHV6, present in more than 90% of infected individuals in the UK. Firstly, PBMC from 14 healthy donors were stimulated in-vitro with 10 pools containing five U90 peptides predicted to bind to HLA class I alleles A1, A2, A24, B7 and B8, and IFNγ responses detected by ELISPOT. No responses were observed using this approach, suggesting that U90 was perhaps not strongly immunogenic in-vivo. This analysis involved a limited set of peptides and was restricted to a limited set of class I HLA types. Thus, we switched to a broader approach using whole antigen peptide mixes. PBMC from a further 30 healthy donors were stimulated with peptide-mixes corresponding to U90 and additional HHV6B antigens U54, U11 and U39. We observed CD8$^+$ and CD4$^+$ responses to U90 and U54 respectively in a number of donors, but in comparison to other control virus antigens, these responses were significantly lower in magnitude and frequency.

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Safety, immunogenicity and reactogenicity of recombinant hepatitis B vaccine Engerix™-B

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About 2 billion people are infected with Hepatitis B virus worldwide; more than 350 million of them are chronic hepatitis B carriers. Chronic infection with HBV can lead to severe medical outcomes, including cirrhosis, hepatic failure, and hepatocellular carcinoma. Countries in South-East Asia have historically been regions of high HBV endemicity In Pakistan the HBV carrier rate is about 10–11%. Hepatitis B vaccination is one of the most effective strategies for preventing hepatitis B infection.

Main objective of this study was to assess the safety, reactogenicity and immunogenicity of adjuvanted hepatitis B vaccine, Engerix™-B.

A total of 400 subjects were taken, from the Rawalpindi, Pakistan. The serum samples were analyzed by Enzyme Linked Immunosorbant Assay (ELISA) for the quantitative determination of antiHBs antibodies. The prevaccination results served to make a baseline. As 88 (22%) subjects out of the total 400 were found to be seropositive at baseline. The post vaccination results showed that about 15 (7.53%) of subjects had inadequate levels of antibodies (i.e. <10 IU/l). Overall 92.46% subjects showed a positive response to the vaccine. Immunization was well tolerated by all the subjects, and no serious adverse event was reported. In conclusion, this prospective study reinforces that the Engerix™-B Hepatitis B vaccine used in this study has a good tolerability and is highly immunogenic among infants. It is recommended that serosurvey of HBsAg and vaccine coverage at country level should be done.

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Early events in cutaneous delayed type hypsersensitivity response in ageing

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Immunity declines during ageing and reactivation of varicella zoster virus (VZV) causing shingles is more common in the elderly. We use a human cutaneous model to compare the ability of old (>65 years) and young (<40 years) individuals to mount a delayed type hypersensitivity response. We have previously shown that responses to recall antigens such as tuberculin purified protein derivative, *Candida albicans* and VZV are significantly reduced in older individuals.

After intradermal VZV antigen challenge we have found that there is significantly reduced infiltration of CD4+ and CD8+ T cells, as well as CD11c+ dendritic cells in old skin compared to young. This is probably due to a lack of recruitment of these cells from the circulation as the dermal endothelium is less activated in the old compared to young at 24 h (>70% and <20% of CD31+ capillary loops express E-selectin respectively) after antigen challenge. We hypothesised that there is a defect in the innate immune response to antigen challenge in the old contributing to defective activation of endothelium at 24 h. Interestingly, 6 h after VZV antigen challenge, there was no difference in endothelial activation (25% of CD31+ dermal vessels expressed E-selectin), or recruitment of neutrophils, which are commonly involved in innate responses, between old and young skin. Therefore, early activation of dermal endothelium is not impaired in old skin but rather there appears to be a failure of subsequent, perhaps antigen specific, amplification of early activating signals.

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Discovery and characterization of $CD4^+$ cells in zebrafish (*Danio rerio*)

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CD4 T-helper (Th) cells are important in adaptive immunity, regulating the different types of immune responses that are required to combat bacterial, viral and parasitic diseases. The CD4 molecule is a transmembrane protein expressed on the surface of Th cells, where it functions as co-receptor with T cell receptor (TCR), by binding to the antigen loaded major histocompatibility complex (MHC) class II molecules. Although CD4 is known to exist in fish, the role of CD4+ cells within the adaptive immune response has not been investigated. In this study, we focus on characterising the CD4+ cells to help understand their role in teleost fish. Zebrafish appears to be unique as they have four CD4 molecules within its genome, compared to two found in other teleosts and one found in mammals. They are located on Chromosome 16 and have different number of amino acid and Ig like domains. And rabbit polyclonal antibody against two peptides of one of zebrafish CD4+ cell (CD4L) was designed for further study. Western blot analysis using the anti zebrafish CD4 antibody has detected an immunoreactive protein bands with a calculated mass of approximately 52 kDa which matches well with the predicted theoretic mass. The CD4+ cells accounts for approximately 2% of the total leucocytes isolated from zebrafish kidney. Real-time PCR analysis shows that the CD4 molecules are moderately expressed in the lymphocyte population rather than the monocytes and other cell types. The functions of the CD4+ cells are currently being investigated in various zebrafish disease models.

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Ability of different isolates of *Staphlococcal aureus* to induce selected metabolites in human polymorphonuclear leucocytes

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Staphylococcus aureus is a gram positive bacterium that causes a number of diseases such as abscesses, infective endocarditis, septic arthritis, etc. It is acquiring resistance against many antibiotics like methicillin due to which its control is becoming difficult. Peripheral blood phagocytes play an important role in the protective mechanisms against these organisms. Phagocytes interact with bacteria and phagocytose them. The focus of this study is to test the hypothesis that different isolates of *S. aureus* induce the production of ROI and RNI differently and it may correlate with their antibiotic resistance. For this one hundred different isolates of *S. aureus* were obtained from various hospitals of Lahore in which fifty isolates were methicillin resistant while rest of the fifty isolates were methicillin sensitive. Peripheral blood from healthy individuals was used to obtain polymorphonuclear leucocytes by dextran density gradient centrifugation. Polymorphonuclear leucocytes were exposed to bacterial strains at 37°C in the presence of opsonin. Microbiological methods were used for the determination of phagocytic index of these isolates. Superoxide concentration was determined by the reduction of ferricytochrome c and nitric oxide concentration was measured by Griess method. Methicillin sensitive *S. aureus* (MSSA) has showed more phagocytosis by human polymorphonuclear leucocytes as compare to methicillin resistant *S. aures* (MRSA). Similarly, a significant difference was observed between two isolates of *S. aures* to induce ROI and RNI by human PMNs. MRSA produced more mean value of superoxide and nitric oxide as compare to MSSA.

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Establishing a T cell assay to evaluate the risk of immunogenicity for therapeutic proteins

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During the last 20 years, biological agents have become very powerful as therapeutic drugs. Despite this progress in the field of therapeutic proteins, immunogenicity (formation of anti-drug antibodies) is still a considerable clinical issue in development of these drugs. The consequences of an immune reaction directed against a therapeutic protein can range from the transient appearance of antibodies with minimal clinical significance to severe life-threatening effects, including allergic reactions, serum sickness and anaphylaxis. An initial step in generation of high affinity anti-drug antibodies is the activation of CD4+ T helper cells. Therefore, *in vitro* assays testing for drug mediated T cell activation can help assess the risk of clinical immunogenicity for a therapeutic protein. T cell activation is commonly determined by measuring T cell proliferation, expression of T cell specific surface markers and also cytokine release. For this purpose, Peripheral Blood Mononuclear Cells (PBMCs) are prepared from the whole blood of selected healthy human donors by density gradient centrifugation. PBMCs are cultured for 7 days. On day 0, cells are treated with different concentrations of therapeutic proteins and IL-7 as a T cell survival factor. After 7 days, cells are harvested and investigated for different immunological parameters. The results from this set of experiments showed an increase in the number of T cells which produce IFN-γ and also T cell proliferation upon treatment with reference proteins including a therapeutic protein. The frequency of T cell activation observed *in vitro* correlates well with clinical immunogenicity incidence reported for the therapeutic protein.

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Pre-clinical immunogenicity 'check up' of biotherapeutic drugs

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The majority of biotherapeutic proteins are, to a variable extent, immunogenic. Clinical immunogenicity can compromise drug safety, alter drug phamacokinetics and reduce efficacy. During the development stage and as soon as possible, a risk assessment and control of the potential immunogenicity is highly desirable before reaching clinical development phases. The value of such pre-clinical assessment using one or a combination of *in silico, in vitro* and *in vivo* immunogenicity predictive tools, has been recognized in the last few years and increasing numbers of biotechnology-derived therapeutics under development are evaluated using these methods.

Intrinsic and extrinsic factors such as T-cell epitope content, protein structure, glycosilation patterns, degradation products, production contaminants, formulation and aggregates can contribute to biotherapeutic drug-induced immunogenicity. Lonza's integrated immunogenicity services address the challenge of product driven immunogenicity through prediction, detection and characterization of product-induced immune responses. Epibase™ is a structure and statistics-based *in silico* platform to identify T cell epitopes in the context of HLA binding specificity and facilitate lead selection. Epibase IV™ evaluates immunogenicity potential of therapeutics *in vitro* by directly measuring T- and B-cell responses in human target cohorts using high quality PBMCs. Multi-parameter flow cytometry and ELISpot technology provide measurement of relevant parameters (expression of activation markers, cell proliferation, cytokine production) that allow characterizing product associated immune responses. Up-to-date, *in vitro* cellular assays are considered to offer one of the closest surrogate markers to address immunogenicity of biotherapeutics in humans in a rapid and cost-effective manner prior to the first clinical application.

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A human genome-wide siRNA screen for novel machinery involved in regulation of cell surface expression of the MHC class I molecules

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Major histocompatibility complex (MHC) class I molecules continuously enter the cells via clathrin-independent endocytic pathway, and in early endosomes, they are sorted either back to the plasma membrane or for lysosomal degradation. The molecular machinery involved post-Golgi trafficking, quality control, and recycling of the class I molecules remains, however, elusive. We performed a human genome-wide siRNA-based screen for novel machinery regulating cell surface expression of clathrin-dependent and -independent proteins. A fluorescence plate reader-based assay was used to quantify the cell surface levels of MHC class I and of CD8 chimeras with two different clathrin-dependent trafficking motifs, YXXΦ and FXNPXY. The DNA stain Hoechst was used to control for effects on cell viability. We identified 250 hits that specifically increased (and 100 hits that decreased) cell surface levels of MHC class I, and not of the clathrin-dependent cargo proteins. Pathway and Gene Ontology term analysis were performed to group the targeted genes into functional clusters. Among the hits, we found several genes involved in post-Golgi protein trafficking. Interestingly, 30% of the candidate genes involved in regulation of MHC class I have been classed as uncharacterized (11% for clathrin-dependent cargo proteins). Validation and further characterization of the hits should improve our understanding of the mechanisms controlling cell surface expression and turnover of the MHC class I molecules.

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Structural basis of antigen recognition by an archetypal murine T cell ceceptor

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Cytotoxic T cell responses are governed by the presentation of antigenic peptides by class I MHC molecules. H2-K^b is an important murine class I MHC allele, as it has been used in complex with the OT-1 TCR to study the kinetic basis of the TCR-pMHC interaction. However, only a limited number of OT-1 TCR ligands have been described at a structural level. To further this knowledge, we determined the high-resolution crystal structures of agonist (H2-K^b/SAINFEKL) and weak agonist (H2-K^b/EIINFEKL) OT-1 TCR ligands. Soluble forms of H2-K^b (heavy α chain and β2M) were expressed, refolded and crystallized in complex with ovalbumin-derived peptides (SAINFEKL and EIINFEKL). The structures of the H2-K^b/SAINFEKL and H2-K^b/EIINFEKL were solved to 1.86 Å and 1.75 Å resolutions, respectively. Overall, the structures were similar to other reported peptide-H2-K^b complexes, whereby the deep C pocket of H2-K^b bound phenylalanine as the main anchor residue at peptide position 5. Both peptides adopted a zig-zag conformation in the binding groove, which exposed several residues that could be recognized by the OT-1 TCR. The conformation of the exposed asparagine residue at position four differed between the agonist and weak agonist ligands, indicating that the residue is likely to play an important role in OT-1 T cell activation. Finally, SAINFEKL formed fewer contacts with the binding groove compared to EIINFEKL, resulting in peptide flexibility which could contribute to the enhanced ability of H2-K^b/SAINFEKL to activate OT-1 T cells. These findings indicate that subtle differences in peptide conformation may influence T cell recognition.

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Kinetics and antigen specificity of $CD4^+$ memory and regulatory T cells during cutaneous immune responses to *Varicella zoster*

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Delayed type hypersensitivity responses can be induced by intra-dermal injection of recall antigens, in individuals who have a history of previous Ag exposure. We studied the kinetics of the cutaneous VZV response in young subjects (<40 years old) who were challenged with a VZV skin test antigen. We found that although both $CD4^+$ and $CD8^+$ T cells accumulated after VZV injection, only the $CD4^+$ population undergoes significant proliferation. Using a class II tetramer that identifies DR15 restricted IE63 peptide epitope specific $CD4^+$ T cells, we found that that up to 12% of $CD4^+$ cells that were isolated after VZV challenge in the skin were specific for this antigen compared to <0.1% in the blood. Similarly, intracellular cytokine staining showed a significant accumulation of VZV-specific $CD4^+$ T cells secreting IFN-γ, IL-2 and IL-17 in the skin following VZV injection. We observed that Foxp3 expressing, regulatory $CD4^+$ T cells accumulate and proliferate in parallel to the memory $CD4^+$ T cells and represent approximately 10% of total CD4 population, throughout the response. Furthermore a proportion of $Foxp3^+$ cells (1−7%) are also tetramer positive, supporting the possibility that some regulatory T cells may arise as a result of activation of memory T cells. Interestingly some tetramer positive $CD4^+$ T cells can also be identified in normal, non-injected skin suggesting that these VZV specific cells may have a role in immuno-surveillance and could potentially prevent reactivation of VZV and development of shingles in young healthy individuals.

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Peripheral T cell activation and differentiation in elderly melanoma patients

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Age is a negative prognostic factor in melanoma but the immunologic reasons for this are poorly understood. Tumour burden may induce accumulation of senescent or exhausted T cells, as prolonged antigenic burden can induce T cell differentiation towards an end stage, especially in older individuals. This study thus aimed to investigate the effect of melanoma burden on T cell differentiation in old patients (65 year or older; $n = 35$) compared to age matched healthy controls ($n = 25$). T cell differentiation (CD45RA/CD27 expression) and CMV responses (intracellular IFNy expression following overnight incubation with CMV lysate) were assessed by flow cytometry. Compared to healthy controls, Melanoma patients had an inverted CD4:CD8 ratio. In addition, the proportion of Naïve ($CD45RA^+CD27^+$) CD4 T cells decreased ($P < 0.05$) while central memory ($CD45RA\text{-}CD27^+$) T cell increased compared to controls ($P < 0.05$). In the CD8 T cell compartment, the percentage of central memory decreased, whilst effector memory CD8 T cell (CD45RA−CD27−) and effector memory cells re-expressing CD45RA (EMRA; CD45RA+27−; $P < 0.05$) increased significantly. These trends were most prevalent in advanced stage melanoma patients, whereas stage I patients' lymphocyte profiles resembled those of healthy controls. CMV responses were similar between patients and controls, and the melanoma associated differentiation patterns in both T cell groups seemed to be CMV independent. In summary, T cell differentiation appears increased in old melanoma patients, particularly in later disease stages. We are currently investigating the functional characteristics of the highly differentiated T cells in patients with melanoma.

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Orientation of the reading frame of a gene is swapped on DNA replication, pattern of resulting protein is altered, could explain cells become resistant to infection

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The DNA polymerase -I, has a unique ability to start replication *in vitro* at a nick in DNA. At a point where phosphodiester bond has been broken in a double stranded DNA, the enzyme extends the 3′-OH end.

It allows radioactively labeled nucleotides to be inserted in to DNA *in vitro* from present literature.

This data is it self impose a segment of DNA is transfered to new synthesis from parental strands when strands are cleaved for genomic replication.

Replication apratus shift occurs to the other proportion for DNA polymerase which can only extands preveously proportion, since no enzyme is known for replication apratus alratus synthesis. inter shift findings in DNA duplexcould change the shape of paterned receptor and cell would become resistant to parasites and viral inpanetration due to such their altered receptor.

Data also comes from - RNA is taxed, altering its secondary structure. This new secondary structure in some way prevents the cleavage reaction, although the basis of this effect is unknown. Postulating, this might be relating with DNA-base fragment interchange defence system.

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The immunoglobulin heavy-chain locus 3′ regulatory region (hs3a, hs1,2, hs3b, hs4) is dispensable for VDJ assembly

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The immunoglobulin heavy chain locus undergoes numerous genomic rearrangments during B cell maturation (VDJ recombination, class switch recombination, somatic hypermutation). These differents events are regulated by several *cis*-regulatory elements. A 3′ regulatory region (3′RR) located downstream the IgH locus has been shown to contain four lymphoid-specific transcriptional enhancers: hs3a, hs1,2, hs3b and hs4. To understand the role of this 3′RR in B-cell development, the laboratory created a knock-out murine strain deficient for the whole 3′RR (3′RR deficient mice). Previous results shown that the complete 3′RR deletion dramatically affects class switch recombination and Ig secretion for all isotypes (Vincent-Fabert et al, Blood 2010 116: 1895−1898). We use this murine model to investigate the role of the 3'RR in V(D)J recombination. We firstly tested the 3'RR implication in the choice of a specific IgH allele in heterozygous mice. Results demonstrated, in $IgMa^{\Delta 3'RR}$/IgMb animals, the similar use of either 3'RR-deleted allele or wild-type allele for IgM synthesis in bone marrow. The diversity of rearrangement as well as the V, D and J usages were, in $CD25^+$ pre-B cells or mature splenocytes, not affected by the 3'RR deletion. Taken together, these results reveal no evident role for the 3'RR during V(D)J recombination process. Currently, studies are in progress to investigate the role of the 3'RR in somatic hypermutations.

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Generation of inflammatory dendritic cells from lung derived non-classical monocytes

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Aims: Chronic lung rejection occurs partly via allorecognition of donor antigen by recipient dendritic cells (DC). The source of DC in this setting remains unclear, but generation may occur following infiltration of the donor lung by recipient non-classical monocytes (NCM), which then differentiate to DC. The aim of this study was to assess if peripheral NCM migrate into the lung and if differentiation to inflammatory DC occurs.
Methods: Peripheral blood ($n = 60$) and bronchoalveolar lavage (BAL, $n = 20$) samples from lung transplant patients were assessed for DC and NCM using flow cytometry. Isolated NCM were cultured and differentiated to DC, before being immunophenotyped and cytokine profiling performed ($n = 10$). An *in vitro* air-liquid interface lung model was used to evaluate NCM migration and differentiation within the lung.
Results: $CD123^{bright}$ DC increased, and NCM decreased in the circulation in association with chronic rejection. NCM and $CD123^{bright}$ DC were present in all patient lavages. Isolated NCM were capable of generating $CD123^{bright}$ DC *in vitro*, and stimulated cells secreted pro-inflammatory cytokines (IFN- and IL-2). Peripheral blood NCM rapidly diapedesed across the *in vitro* lung model, and when cultured on the alveolar layer, differentiated to $CD123^{bright}$ DC.
Conclusions: We report a divergence of NCM to DC in patients with chronic rejection, and demonstrate the presence of airway NCM. *In vitro*, pro-inflammatory cytokine producing $CD123^{bright}$ DC were generated from NCM following tissue diapedisis into the lung. This has significant implications in the transplant setting, identifying a source of DC which play a major role in graft rejection.

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The role of Nod-like receptors in the host defense against *Salmonella typhimurium*

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Nod-like receptors (NLRs) are cytosolic receptors which play a central role in the host defense against *S. typhimurium*. NLRs recognise specific microbial ligands and trigger the formation of a multi-protein complex known as the 'inflammasome', which consists of caspase-1 and the adaptor protein ASC. In this study, we investigated the role of multiple NLRs in the production of cytokines, macrophage cell death, and in the control of bacterial numbers in macrophages challenged with the bacterial pathogen *Salmonella enterica* serovar Typhimurium (*S. typhimurium*).

We have shown that the NLRC4 and NLRP3 receptors found in macrophages are important for the recognition of *S. typhimurium*. Both receptors required the adaptor protein ASC for efficient IL-1β processing. Indeed, immunofluorescence staining revealed that macrophages infected with *S.* Typhimurium initiated striking re-distribution of ASC and caspase-1 to form a single cytoplasmic focus, providing a site for IL-1β processing. In addition, intracellular delivery of flagellin from *S. typhimurium* into macrophages to activate NLRC4 resulted in ASC foci formation, suggesting that NLRC4-ASC inflammasomes are formed for IL-1β processing. However, macrophage cell death initiated by *S. typhimurium* does not require ASC, a process which is critically dependent on NLRC4 and caspase-1. These results suggest that NLRC4-caspase-1 inflammasomes are formed to drive macrophage cell death independently of ASC. Furthermore, NLRC4 also contributed to the control of intracellular bacterial numbers and distributions in a manner dependent on caspase-1, but not NLRP3 or ASC.

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Innate immunity in dental pulp

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Toll-like receptors (TLRs) are essential for the innate immune responses to the wide range of pathogens. TLR2, 3 and 4 are crucial for the recognition of peptidoglycan, genomic RNAs and Lipopolysaccharide, respectively. Dental pulp is a unique connective tissue that expresses TLRs. This study investigates the expression of TLRs in premolars and molars teeth and the effects of amalgam restorations on the expression of these genes in premolar ones.

Ten Intact human molar and premolar teeth plus 5 premolars with amalgam restorations were obtained. RT-PCR and QPCR were used to show and compare the expression of TLR2, three and four genes in these teeth.

TLR2-4 genes were expressed both in human molar and premolar teeth with or without amalgam restorations. QPCR has shown less expression of TLR2-4 in premolars in comparison with molars. Meanwhile, less expression of these genes were seen in molar teeth with amalgam restoration in comparison with controls.

Interestingly, this study revealed that localization and anatomical differences in premolar and molar teeth might provide different innate immune responses through TLRs against related pathogens. Besides, amalgam restorations might have immunosuppressive effects on premolar teeth by reducing the expression of TLR2-4. Consequently, this makes teeth susceptible to infectious agents.

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Co-ordinated crosstalk of human γδ T cells, neutrophils and monocytes in response to bacterial infections

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Human Vγ9/Vδ2-T-cells constitute 1–5% of all peripheral blood T-cells under normal conditions but can rapidly increase to over 50% in acute infection. The direct recognition of invading pathogens by Vγ9/Vδ2-T-cells is attributed to their unique TCR-mediated detection of the microbial metabolite, HMB-PP. We have identified a crucial role played by infiltrating neutrophils in their ability to facilitate the release of functional HMB-PP from phagocytosed bacteria. Neutrophils harbouring HMB-PP over-expressing but not HMB-PP deficient bacteria induced potent γδ-T-cell cytokine responses (IFN-γ and TNF). Transwell experiments showed that γδ-T-cells responded directly to soluble HMB-PP released from infected neutrophils and that cell-cell contact with monocytes was required for optimum activation. We observed this crosstalk with autologous γδ-T-cells, neutrophils and monocytes in response to HMB-PP producing (e.g. *Klebsiella, Enterobacter, Pseudomonas*) and HMB-PP deficient live bacterial pathogens (e.g. *Staphylococcus, Enterococcus, Chryseobacterium*). Monocytes provide a key presenting mechanism for microbe derived HMB-PP whereby they take up and directly present this activator to induce γδ-T-cell responses; this could be blocked by the use of various inhibitors of monocyte endocytosis. Microbe responsive γδ-T-cells also interacted with neutrophils by providing potent long-lived survival and activation signals. We have also observed similar activation marker expression on neutrophils and γδ-T-cells in patients with sepsis. Taken together our findings define an unconventional mechanism of pathogen recognition, which links the crucial innate function of pathogen clearance by neutrophils with an alternative antigen processing pathway in monocytes, resulting in the highly co-ordinated activation of γδ-T-cells and potentiation of the cellular immune response.

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The role of MAPK signalling in the regulatory phenotype of macrophages in response to *Schistosoma mansoni* cercarial secretions

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The interaction between antigen presenting cells in the skin with molecules secreted by cercariae of *Schistosoma mansoni*, one of the pathogens that cause Schistosomiasis, constitutes the first point of contact between the host and the pathogen. Macrophages (MΦ) have been shown to play a pivotal role in modulating the immune response to parasites, and in the skin, they are amongst the cell populations to take up these secretions. Both *in vitro* and *in vivo* MΦ produce cytokines when exposed to the parasite released products, but it remains unclear what signalling pathways are being activated by secreted antigens. Evidence suggests that Toll-like receptors (TLRs) play a role in the process, however which receptors are engaged and what molecules are activated downstream of the receptors remains to be elucidated. The aim of this study is to identify the signalling pathway(s) triggered in MΦ by molecules secreted by cercariae within 3 h of skin invasion. We have determined that multiple MAPK pathways are activated and control cytokine production in response to the secretions. Experiments selectively inhibiting these pathways have allowed us to partially determine the individual contribution of each signalling cascade. Our results suggest that the activation of the MEK/Erk pathway is important for the regulatory phenotype of the MΦ. Inhibiting this pathway results in partially blocking the production of IL -10, whilst significantly increasing the levels of IL-12. Ongoing studies seek to identify the receptor responsible for the activation of the MEK/Erk pathway, and subsequent validation of the results *in vivo*.

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Influence of breast cancer cell-derived factors on the phenotype and cytotoxicity of human $CD56^{low}CD16^{+}$ and $CD56^{high}CD16^{-}$ NK cell subsets

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Rationale and hypothesis: Although tumours can circumvent protective anti-tumour immunity, many of the mechanisms remain unclear. Growing evidence indicates that natural killer (NK) cells in patients with cancer exhibit different surface receptor profiles to those of healthy controls, yet there is little insight into the capacity of tumours to directly influence the NK cell phenotype and function.
Objectives: To examine the influence of human breast cancer cell-derived factors on the expression of activation and inhibitory receptors by NK cell subsets and their cytotoxic potential.
Methodology: NK cells were isolated from human PBMCs and cultured (2×10^5cells/well) for 24 h in the presence/absence of supernatants from MDA-MB-436, MCF-7 and T47D human breast cancer cells (50% v/v) and/or IL-2 (100 U/ml). NK cell cytotoxicity was assessed by incubating NK cells for 3 h at 5:1 ratio with K562 or MDA-MB-436 cells and analysing target cell death using flow cytometry. Surface expression of CD94, CD69, NKp46, NKG2D and CD158e1 by $CD56^{low}CD16^{+}$ and $CD56^{high}CD16^{-}$ NK subsets was determined by flow cytometry pre- and post-incubation.
Findings: All supernatants showed the capacity to increase CD158e1 and decrease NKG2D expression but in a volunteer-dependent manner, while CD56 and CD16 expression was reduced in all volunteers. Only MDA-MB-436 supernatant inhibited killing of K562 target cells by IL-2 activated NK cells, but it had no effect on the killing of MDA-MB-436 cells.
Conclusions: Breast cancer cell-derived factors can modify NK cell receptor expression and influence NK cytotoxicity in a volunteer-dependent manner.

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The role of innate interferon responses during pulmonary viral infections

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Type I and III interferons (IFN) are the first line of defense against viral infections. They play an important role in the induction of antiviral responses and at the same time in the activation of innate and adaptive immune responses, crucial to protect from virus spread and to limit disease. Viral respiratory infections are the leading cause for hospitalisation in early childhood. Respiratory syncytial virus (RSV) can cause severe bronchiolitis in infants and young children and RSV bronchiolitis have been associated with polymorphisms in several innate immune response genes, in particular many that control the interferon (IFN) system. However, the role of these cytokines in regulating host resistance and immunopathology during RSV infection in young and adults is poorly understood. We elucidate the type I and type III IFN production and the induction of interferon stimulating genes (ISGs), in neonates and adults using a mouse model for RSV infection. Preliminary results show a lower production of IFNs in neonates compared to adults after RSV infection. We are currently exploring the role of this defect in the control of the infection and immunopathology.

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***Mycobacterium bovis* BCG binds factor H**

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Bacteria and other microorganisms have evolved many mechanisms for evading the host immune system. Some have developed the ability to down-regulate complement activation by utilizing complement regulatory proteins such as factor H (FH (Diaz et al., 1997; Meri et al., 2002; Schneider et al., 2006; Hallstrom et al., 2008). *M. tuberculosis*is is believed to evade the immune system by actively gaining entry into phagocytes, and surviving intracellularly. This study explores the binding of FH to *M. bovis* BCG. Results confirmed previous findings that *M. bovis* BCG binds FH in the presence of EDTA. In addition, it was determined that there were greater binding in the presence of Ca^{2+} Mg^{2+}than in EDTA. Binding in EDTA was optimal at physiological salt and in divalent metal ion (M^{++}), binding was optimal at low salt while both binding conditions had low pH optima of 5–6. We tested for binding specificity of FH and results suggest other plasma protein(s) compete for FH binding sites on the mycobacterial cell in the presence of M^{++} while no other serum proteins compete for FH binding sites in the presence of EDTA. Competition assays between FH, C1q and B_2glycoprotein 1 show that these serum proteins do not compete for FH binding site. Furthermore FH was shown to effect macrophage uptake of *M. bovis* BCG. This study also attempts to determine FH domains that mediate binding to mycobacteria.

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Sphingosine regulates the NLRP3-inflammasome and IL-1β release from macrophages

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Interleukin-1β (IL-1β) is a pro-inflammatory cytokine that regulates inflammatory responses to injury and infection. IL-1β secretion requires the protease caspase-1, which is activated following recruitment to inflammasomes. Endogenous danger associated molecular patterns (DAMPs) released from necrotic cells activate caspase-1 through a NLRP3-inflammasome. Here we show that the endogenous lipid metabolite sphingosine acts as a DAMP by inducing the NLRP3-inflammasome-dependent secretion of IL-1β from macrophages. This process was dependent upon serine/threonine protein phosphatases since the PP1/PP2A inhibitors okadaic acid and calyculin A inhibited sphingosine-induced IL-1β release. IL-1β release induced by other well characterized NLRP3-inflammasome activators, such as ATP and uric acid crystals, was also blocked by these inhibitors. Thus we propose sphingosine as a new DAMP, and that a serine/threonine phosphatase (PP1/PP2A)-dependent signal is central to the endogenous host mechanism through which structurally diverse DAMPs converge on NLRP3-inflammasome activation.

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TREM-2: expression in non-myeloid cell types and regulation by inflammatory mediators

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The triggering receptor expressed on myeloid cells (TREM) family include TREM-1, TREM-2 and TREM-3. Whilst TREM-1 and -3 are pro-inflammatory, evidence from models of inflammatory disease, such as multiple sclerosis, suggest TREM-2 is in fact anti-inflammatory. TREM-2 is expressed in myeloid cells and is down-regulated in response to the bacterial endotoxin LPS and upregulated by the anti-inflammatory cytokine IL-4. However, the effect of other inflammatory mediators on TREM-2 expression and the mechanisms of regulation remain unknown. This study investigated TREM-2 expression in other immune cell types and examined the regulation of TREM2 by pro- and anti-inflammatory mediators in myeloid cells.

TREM-2 gene expression, measured by qRT-PCR, was highly expressed in monocyte, macrophage and microglial cell lines (THP-1, RAW 264.7 and N9), but unexpectedly was not detected in the promyelocytic cell line, HL-60. TREM-2 mRNA was also expressed in selected innate non-immune cells, including primary human small airway epithelial cells and murine fibroblasts (3T3-L1). In THP-1 and RAW 264.7 cell lines, LPS rapidly down-regulated TREM-2 mRNA expression. This was also observed with other TLR agonists (peptidoglycan, Poly I:C and Pam3Cys) and to a lesser extent with TNF-α. Interestingly, the anti-inflammatory cytokines, IL-4, IL-13 and TGF-β1 increased TREM-2 mRNA expression. These data demonstrate that TREM-2 is expressed in non-myeloid cell types and confirms the ability of anti-inflammatory mediators to up-regulate TREM-2. Combined with current evidence supporting TREM-2 as an anti-inflammatory mediator, this study suggests that TREM-2 may play a role in the resolution of inflammation.

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Suppressors of cytokine signalling (SOCS) are essentail regulators of macrophage polarization with consequential effects on tumour outcome

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Monocytes are recruited to the tumour microenvironment and polarize into macrophages (MØs) which can have a profound impact on tumour growth and are broadly classified into two subsets: (i) Inflammatory, tumoricidal 'classically activated' M1 MØs or anti-inflammatory, pro-tumour 'alternatively activated' M2 MØs. High M2 MØ density in tumours is associated with poor prognosis in patients. SOCS proteins attenuate cytokine signaling via the JAK/STAT pathway, and therefore regulate inflammatory responses. Our findings indicate that deletion of SOCS2 and SOCS3 genes in mice alter MØs subset homeostasis and polarisation towards M1 or M2 subsets, respectively. Opposing MØ subsets observed in these mice resulted in divergent tumour growth rates in both in a syngeneic murine colorectal adenocarcinoma model and in a chemically inducible model of squamous cell carcinoma (SCC) whereby SOCS2 KO mice exhibited resistance to both tumour models whereas mice with the myeloid restricted deletion of SOCS3 were highly susceptible to tumour initiation and growth compared to wild type (WT) mice. Myeloid restricted deletion of SOCS3 in mice results in accelerated tumour growth accompanied by enhanced angiogenesis (CD34 staining), and macrophage infiltration (F4/80, CD11b). These findings suggest that SOCS proteins have a profound influence on macrophage polarization and consequently tumour growth. Targeting SOCS proteins may be a viable therapeutic target in treatment of cancer.

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Using fluorescent labelling of schistosome cercariae to investigate innate immune responses during infection

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Schistosomes are parasitic trematodes that invade their hosts via percutaneous penetration. The immune response during initial invasion is critical to the outcome of infection and of particular interest is how larval parasites (cercariae) interact with innate immune cells, which condition dermal responses and present antigens to the acquired immune system. Labelling cercariae with fluorescent markers provides a novel means of visualising this interaction and has recently been used to show that parasite secretions are internalised by antigen presenting cells (APCs) within hours of infection. However, existing methods using the amine reactive dye carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) are limited by the overlap of its emission spectra with auto-fluorescence in the skin. The purpose of this study is to develop new protocols for fluorescent labelling of cercariae that can be used to track the fate of schistosome antigens and identify the cells that interact with them. Live *Schistosoma mansoni* cercariae were effectively labelled using Far-red DDAO-SE, which has greater photo-stability than CFDA-SE and minimal spectral overlap with other commonly used fluorophores and skin auto-fluorescence. The fluorescence intensity per parasite was determined by fluorimetry and the specificity of labelling for molecules in acetabular glands was validated using confocal microscopy. Subsequent experiments showed that cellular immune responses did not differ between labelled and un-labelled parasites. In addition, the uptake of labelled antigens by APCs was detectable via flow cytometry. The results of this study demonstrate the potential for Far-red DDAO-SE labelled *S. mansoni* cercariae as a tool for future investigations of schistosome immunobiology.

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The role of physical damage in susceptibility to bacterial superinfection following RSV infection in mice

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Respiratory viruses such as respiratory syncytial virus (RSV) are often associated with increased morbidity due to secondary bacterial infections in the lung. Susceptibility to bacterial superinfection may be increased following viral infection by a number of mechanisms including synergism between viral factors and bacterial adhesion, overregulation of innate immunity in lungs resolved of primary viral infection and physical damage caused by the inflammatory response to viral infection. The role of physical damage in bacterial susceptibility following primary viral infection was investigated using an animal model of RSV and *S. pneumoniae* co-infection. Mice were significantly more susceptible to *S. pneumoniae* up to 6 weeks after the initial RSV infection, despite an increased number of leukocytes in the airway. Lung permeability was probed using fluorescently labelled dextran macromolecules and was elevated up to 21 days after RSV infection. Increased expression of endothelial caveolin-1 correlated with increased endothelial permeability, suggesting that transcytosis of macromolecules between blood and airway is up-regulated during RSV infection. The integrity of lung epithelial tight junctions was also diminished during RSV infection. This work demonstrates that epithelial and endothelial damage is apparent 2 - 3 weeks after initial RSV infection, potentially exacerbating bacterial superinfection, but does not account for the long-lived susceptibility to *S. pneumoniae* observed 6 weeks after RSV infection.

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Complement activation and hypercomplementemia in normal pregnancy

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The complement system is involved in the pathogenesis and mechanisms of injury in diseases such as antiphospholipid syndrome and the estrogen-dependent angioedema. Results of the exploration of this system are different from one study to another. The purpose of this study was to assess the variations of components of the classical and alternative pathways as well as to evaluate the activation state of this system during normal pregnancy.

This work has focused on 121 normal pregnant women with gestational ages ranged from 10−36 weeks and 35 non-pregnant women as controls. For each patient we performed the measurement of C3, C4 and C1 inhibitor by nephelometry and factor B by radial immunodiffusion (Binding site). C3a was measured by ELISA (Hycult biotech) and CH50 by classical hemolytic technique.

The results indicated that there is a statistically significant increase of C3, C4, CH50 and factor B for pregnant women when compared with controls ($P < 10^{-3}$, $P < 10^{-8}$, P: 2.10^{-2} and $P < 10^{-6}$ respectively), whereas the C1 inhibitor concentration remain unchanged. In terms of C3a, a significant increase of this product was found for pregnant women ($P < 10^{-6}$).

Our results demonstrate that there is a complement activation in pregnancy with hypercomplementemia which could be due to the presence of an inflammatory background during pregnancy.

In diseases affecting pregnant women, we should be very careful in interpreting the values of complement components and its activation state by referring to standards of the pregnant woman and not those of non pregnant women.

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The effect of vitamin D on monocyte biology: a physiological perspective

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The active metabolite of vitamin D, $1,25(OH)_2D_3$ and activation of its cognate receptor (VDR), have been shown to have wide-ranging effects within the immune system, spanning both innate and adaptive responses. These include important roles in differentiation of immune cells, particularly mononuclear phagocytes; induction of antimicrobial peptides; homeostatic regulation of immune responses through modulation of innate immune signalling pathways and dendritic cell (DC) interactions with T cells. However, the physiological significance of these reports requires further investigation. Vitamin D deficiency is associated with certain infectious and autoimmune diseases, and is defined by serum levels of the precursor of the active metabolite-$25(OH)D_3$. We have tested the effect of $25(OH)D_3$ supplementation on macrophage and DC biology *in vitro,* in comparison to stimulation with $1,25(OH)_2D_3$. We present data on the capacity of each of these cells to convert $25(OH)D_3$ to the active metabolite, and present a physiologically-relevant model for the role of $1,25(OH)_2D_3$ in the generation of 'tolerogenic' dendritic cells. We also show diverse gene expression changes in response to stimulation with $1,25(OH)_2D_3$ that suggest a major contribution by alternative VDR signalling pathways. Our findings extend previous understanding of the effects of vitamin D deficiency and physiologically relevant supplementation on macrophages and dendritic cells.

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Calpain control of *Listeria monocytogenes* phagosomal escape in macrophages

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Macrophages sense infection by detecting pathogen associated molecular patterns (PAMPs). This, activates signalling pathways that induce the synthesis of cytokines, among them the pro-inflammatory cytokines IL-1alpha and IL-1beta, crucial for the host-response and resistance. IL-1beta is synthesised as a precursor molecule that needs to be processed by caspase-1 in order to be released. Calpain is a calcium dependent protease suggested to be important for the cleavage of the precursor form of IL-1alpha. It has not previously been implicated in the release of IL-1beta.

Listeria monocytogenes is a Gram positive facultative intracellular bacterium that can survive and replicate within different cells including macrophages. For its growth, it must escape from the phagosome into the cytosol. This escape is required for inflammasome assembly and IL-1beta secretion after infection.

In the present work we studied the role of calpain in IL-1beta release after *L. monocytogenes* infection using J774 and murine peritoneal macrophages. Inhibition of calpain with MDL28170 impaired the processing and release of IL-1beta induced by *L. monocytogenes,* while it does not affect release of IL-1beta induced by other danger associated molecular patterns (DAMPs) like ATP or MSU. We found that calpain inhibition, with MDL28170 or calpeptin, blocked phagosomal escape of *L. monocytogenes* and consequently their replication within the macrophage. MDL28170 did not affect bacterial viability or the production of virulence factors (such as listeriolysin).

These data suggest that *L. monocytogenes* co-opts host calpain to facilitate its escape from the phagosome and thus may be considered as an anti-virulence drug target.

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CD14++CD16+ monocyte/macrophages can induce fibrosis in human liver

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Background and aims: Liver fibrosis is a wound healing response to chronic liver injury where macrophages and infiltrating monocytes participate in both the development and resolution of fibrosis. The aim of our study is to characterize the different monocyte/macrophage subsets in human liver disease.
Methods: Liver-infiltrating and peripheral blood mononuclear cells (MNC) were isolated from normal individuals or patients with liver disease (ALD, NASH, PSC, PBC, AIH) using Lympholyte. Flow cytometry was used to characterize monocyte subsets and to evaluate the ability of Th1/Th2 cytokines to induce monocyte subset differentiation. Monocyte conditioned-media was added to cultures of primary hepatic stellate cells (HSC). Gene expression changes (aSMA and COL1α1) were assessed by QRT-PCR.
Results: Classical CD14++CD16− monocytes were present in high numbers (80% of MNC) in normal and diseased peripheral blood, but significantly reduced (50%) in both normal and diseased liver. CD14++CD16+ intermediate subset constituted 9% and 14% of MNC in normal and diseased peripheral blood, respectively, but was significantly increased in normal and diseased livers (42% and 30% of MNC, respectively). In-vitro stimulation of peripheral blood CD14++CD16− monocytes with TGFβ1 or IL-10 induced their differentiation into CD14++CD16+. CD14++CD16+ expressed higher levels of CD163 and HLA DR compared to CD14++CD16− subset, colocalized with CD68, secreted IL-6, IL-8 and IL-13 and promoted fibrogenic response in HSC.
Conclusion: Compared with normal livers, diseased livers harbour fewer CD14++CD16− but significantly more CD14++CD16+ monocytes. TGFβ1 and IL-10 present in the fibrotic microenvironment could promote the differentiation of CD14+ into pro-fibrogenic CD14++CD16+ subset, which further activates HSC.

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Pneumococcal carriage following experimental human challenge does not correlate with SLPI or anti-polysaccharide IgG levels in nasal wash

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Introduction and objectives: An Experimental Human Pneumococcal Challenge (EHPC) platform has been developed and can be used as an immunological probe of mucosal immunity. Healthy volunteers are inoculated intranasally with *Streptococcus pneumoniae* and innate factors, humoral and cellular responses are investigated.

Secretory Leukocyte Protease Inhibitor (SLPI) is found in epithelial secretions and can exert anti-microbial actions against Gram positive bacteria, including *S. pneumoniae*. Antibodies to pneumococcal polysaccharide (PS) are protective against colonization. We aimed to determine if SLPI and anti-PS serotype 6B IgG levels in volunteer nasal wash prior to inoculation were predictive of carriage/non-carriage.
Methods: Participants were screened for natural carriage of pneumococcus by nasal wash. If negative, participants were inoculated with *S. pneumoniae* 6B (15 000−60 000 CFU/ml). Carriage was determined by the presence of pneumococci in nasal wash samples at 48 h and/or 7 days post-inoculation. SLPI and anti-PS6B IgG levels in nasal wash were determined by ELISA.
Results: Fifteen participants were inoculated with 6B pneumococcus. SLPI levels in the nasal wash prior to challenge did not predict carriage. In those that carried pneumococci and those that did not, SLPI levels at 48 h post-challenge were similar. SLPI levels did not alter the duration of carriage. No difference was observed in anti-PS6B IgG levels in NW prior to challenge in colonized and non-colonized volunteers.
Conclusions: Pre-challenge levels of SLPI and anti-PS6B IgG in nasal wash do not predict if carriage will occur following inoculation with 6B pneumococcus. SLPI levels in nasal wash do not alter pneumococcal carriage.

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Differential micro-RNA expression in human blood monocyte subpopulations

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Two main human blood monocyte subpopulations, $CD14^{+}CD16^{-}$ and $CD14^{+/lo}CD16^{+}$, have been described with numerous phenotypic and functional differences, however the role of micro-RNA (miRNA) in monocyte subpopulation is still unexplored. In this study, we specifically investigated the miRNA that are differentially expressed by these two monocyte subpopulations. Twenty-one differentially expressed (DE) miRNAs were identified using Illumina miRNA arrays of which 12 miRNAs had higher expression in $CD14^{+/lo}CD16^{+}$ monocytes and 9 miRNAs had higher expression in $CD14^{+}$ $CD16^{-}$ monocytes. Functional classification of both predicted target genes (obtained from any two combination of five target prediction programs) and validated target genes (obtained from Argonaute database) regulated by the DE miRNAs indicated these genes to be mainly involved in inflammatory response, cell death, cellular development, cellular movement and cell-to-cell signaling and interaction. Interestingly a large number of the target genes regulated by the miRNA more highly expressed in the $CD14^{+/lo}CD16^{+}$ monocytes were associated to cell death. In particular, we could validate experimentally the association of miR-132 with apoptosis in monocytes and this is in agreement with our previous study showing that $CD14^{+/lo}CD16^{+}$ monocytes is more susceptible to spontaneous apopotosis. In summary, for the first time, we identify differential miRNA expression in human blood monocyte subsets. Our data suggest that potential targets of those miRNAs play an important role in the functional differences between $CD14^{+}$ $CD16^{-}$ and $CD14^{+/lo}CD16^{+}$ monocyte subpopulation and manipulation of miRNAs may gain further insight into human monocyte heterogeneity.

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Aluminium adjuvants potently inhibit the secretion of IL-12 which may explain their poor efficacy in promoting Th1 responses

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Alum, a common name for a number of aluminium-containing adjuvants, is the most common adjuvant in clinical use. It has a record of successful use in vaccines, where antibody-mediated immune responses can confer protective immunity e.g. diphtheria, tetanus and hepatatis B. However alum is a poor inducer of cellular immune responses and is not efficient in vaccines for diseases such as tuberculosis, HIV or malaria where Th1 responses are required. Recent studies have shown that the adjuvant properties of particulate adjuvants including alum can be enhanced by incorporating additional immunostimulants. One such approach is to use combinations of Toll-like receptor (TLR) ligands with alum. TLR ligands including LPS and CpG are effective Th1 promoting adjuvants, because they induce IL-12 secretion by innate immune cells. However, we have found that alum inhibits the induction of IL-12 by TLR ligands in dendritic cells (DC). Alum selectively inhibited expression of the IL-12 p35 subunit and the inhibitory effect was the result of alum-induced PI3 kinase signaling. Moreover, IL-12 inhibition was not restricted to aluminium-containing adjuvants, but was also seen with other widely used particulate adjuvants including calcium phosphate and biodegradable PLG microparticles. Remarkably, the endogenous danger signal uric acid, whose release is promoted by alum, was also a potent inhibitor of IL-12 secretion by DC. Our results strongly indicate that alum is not a suitable platform for the development of Th1 promoting adjuvants because of its potent inhibitory effect on IL-12.

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Human CD117-positive and CD117-negative NK cell subsets

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NK cells are important participants of innate immunity. Generally accepted classification of NK cells stipulates the separation according to fluorescence intensity of CD56 markers on cell surface. The aim of our work was the isolation of these NK subsets based on CD117 marker of CD56 bright NK cells as a special marker to discriminate between two NK cell subsets. After purification of NK cells and separation into two subsets by using CD117 microbeads, we obtained CD117 positive and CD117 negative cells. To find distinctive features of these NK cell subsets, we investigated CD56, CD16, perforin, granzim B, CD44, CD62L, CXCR4, NKG2D, NKG2A, CD210w expression in NK cells from seven healthy donors by flow cytometry, the IL-10 production in ELISPOT assay and cytotoxic activity. We anticipated that CD117 would be associated with CD56 bright cells but found significantly high CD56 and CD16 expression on the fresh isolated CD117-negative cells. It turned out that only CD117-positive NK cells secreted IL-10. In addition, cytotoxic activity toward K-562 cells was higher in CD117 positive subset. We noticed that CD56, CD16, perforin, IFNg were expressed much more in CD117 positive fraction after 18 h incubation in the presence and absence of K562. Actually, the results of the experiments offered an explanation of higher cytotoxity of CD117 positive fraction. We concluded that these two subsets are different in functional activity and CD117 as well as CD56 is marker of cell activity. CD117 positive subset has high cytotoxic, secretory activity unlike CD117 negative fraction.

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The effect of cytokines on porcine monocytes and alveolar macrophages

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Scavanger receptor CD163 and sialic-acid binding lectin CD169 have recently been identified as the key surface molecules involved in the first steps of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) infection, an important porcine pathogen which replicates in porcine myeloid cells. Freshly isolated $CD14^+$ monocytes, however, are considered unsuitable for PRRSV culture and so porcine alveolar macrophages (PAM) are routinely used for PRRSV propagation.

The aim of this study was to identify cytokines which could modulate monocytes to render them susceptible to infection and compare them with PAMs. Firstly, freshly isolated monocytes and PAMs were cultured with various concentrations of huM-CSF, or poGM-CSF. M-CSF enhanced the viability of monocytes and PAMs, and also induced proliferation particularly of monocytes. Furthermore, growth of PRRSV was supported in monocytes cultured for 48 h with M-CSF, and also in unstimulated monocytes. Interestingly, cultured monocytes were able to propagate PRRSV almost to the same degree as PAMs.

Therefore we initiated a targeted search for factors that were known to upregulate/sustain CD163 on monocytes/macrophages and consequently may possibly enhance viral replication. Pro-inflammatory cytokines (IL-1β, IL-6 or TNFα) and macrophage activating factors (LPS, IL-4 or IFNγ) did not have a significant effect on CD163 expression on monocytes, and in the case of IFNγ and IL-4, CD163 expression was reduced. The most significant up-regulation of CD163 was observed with IFNα and IL-10. This is in line with previous observations in human monocytes, and is consistent with the biology of PRRSV where some strains are known to induce IL-10.

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The inflammatory cytokine IL-18 induces self-reactive antibody responses regulated by NKT cells

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IL-18 is one of the mediators produced after inflammasome activation and it is elevated in sera from patients suffering from autoimmune and allergic disease. In mice, repeated injections of IL-18 gives rise to a rapid IgE response within 10 days, implying involvement of innate type responses. We thus set out to investigate the B cell activation in IL-18 injected mice and how this is regulated by iNKT cells. Analysis of antibodies in serum revealed that, in addition to increased IgE levels, IL-18 injected mice also had increased levels of total IgM and IgG with expansion of the natural reactivities NP (4-hydroxy-3-nitrophenyl), PC (phosphoryl-choline) and DNA. We found the innate B cell subset, marginal zone B cells (MZBs) to be involved in the IL-18 induced antibody response, as the response was delayed in MZB-deficient (CD19−/−) mice and the MZB population expanded after IL-18 injections. Histological examination of the spleen of IL-18 injected mice showed that the antibody producing cells were located in $CD138^+$ extra-follicular foci in the red pulp, a mechanism frequently associated with autoreactive responses. When iNKT cell deficient mice (Jα18−/− and CD1d−/−) were injected with IL-18, both the serum antibody levels as well as the formation of extrafollicular foci in the spleen were increased compared to wild type mice. We conclude that elevated levels of IL-18 cause MZBs to become activated and produce autoreactive antibodies in extrafollicular foci and that this process is regulated by iNKT cells.

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C3a drives TH17 lineage decisions in humans via induction of IL-1β production in monocytes

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IL-1β is among the most potent pro-inflammatory cytokines and mediates important immune functions. It is therefore also a therapeutic target in several chronic inflammation and autoimmune states. Monocytes/macrophages are the major IL-1β sources. IL-1β secretion by these cells requires TLR (LPS) and P2X7-receptor (ATP) signals, which in turn activate the inflammasome. However, how exactly LPS signals and ATP availability are regulated during monocyte activation is unclear and the requirement for a second danger signal has long been proposed. Considering the importance of anaphylatoxins in innate immunity, we hypothesised that they participate in IL-1β-production.

Indeed we observed that both, LPS and C3a are absolutely required for IL-1βproduction in human macrophages while in monocytes, C3a increased LPS-induced IL-1β dramatically. Neither C3adesArg, nor C5a showed any effect on IL-1β production. Mechanistically, C3a drives IL-1β production by controlling the release of intracellular ATP into the extracellular space via regulating the function of the ATP-releasing channel pannexin.

Importantly, we found that C3a/LPS-stimulated monocytes induce strong Th17 cell induction in *in vitro* cultures. Thus, our data indicate that C3aR-mediated signalling events are a vital component of the IL-1β/Th17 axis in humans. We are currently assessing if this pathway or its deregulation contributes to IL-17-driven disease states such as rheumatoid arthritis or asthma.

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Response to fungal pathogen associated molecular patterns (PAMPs) at the maternal fetal interface

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Approximately 10% of all births occur prior to term (37 weeks of gestation), accounting for around 70% of perinatal mortality and nearly 50% of long-term neurological morbidity. Numerous factors have been linked to spontaneous preterm labour; intrauterine infection has emerged as a key area of interest, calculated to contribute to up to 40% of cases. Yeast infections are a common type of vaginal infection in pregnant women, caused by fungi of the *Candida* family. The innate immune system utilises multiple pattern recognition receptors (PRRs) in the defence against pathogens. Recognition of fungal pathogen associated molecular patterns (PAMPs) involves numerous PRRs including Toll-like receptors, NOD-like receptors and C-type lectin receptors. Dectin-1 is the major receptor for the recognition of beta-glucan in fungal cell walls. Therefore, the expression and activity of Dectin-1 was examined in gestation associated tissues (placenta, choriodecidua, amnion) collected form healthy term newborns (>37 weeks gestation) delivered by elective caesarean section. Transcripts for Dectin-1 are expressed in all three tissues. Production of the cytokines IL-6, IL-10, and MCP-1 in response to beta-glucan zymosan (recognised by TLR2/6 & Dectin-1), occurs in placenta, choriodecidua amnion. However in response to the zymosan derivative depleted zymosan (recognised by Dectin-1 only), only MCP-1 production by the choriodecidua occurs. Therefore the choriodecidua, may play an important role in defense against ascending fungal vaginal infections.

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ANCA, through the activation of PI3Kγ and MTOR, promote neutrophil exocytosis and inhibit autophagy *in vitro*

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The dysregulated release of both serine protease and myeloperoxidase from the azurophilic granules of neutrophils causes increased tissue damage and amplified inflammation in autoimmunity. We investigated a role for PI3Kγ in auto-antibody induced neutrophil exocytosis. Neutrophils incubated with the PI3Kγ inhibitor, AS-46030 (4 μM), prior to treatment with Anti neutrophil cytoplasmic antibody (ANCA) (200 μg/ml), exhibited a reduced capacity to release myeloperoxidase independently of phosphatidic acid production, demonstrated by a specific release assay and flow cytometry. Confocal microscopy revealed that AS-46030 inhibited translocation of granules to the plasma membrane by inhibiting polymerisation of F-actin in ANCA stimulated neutrophils indicating a distinct mechanism of action. We next investigated the ability of PI3Kγ to promote the activation of MTOR, an important regulator of autophagy in cells. Incubating neutrophils with the MTOR inhibitor rapamycin (100 nM) significantly reduced the ability of ANCA to promote exocytosis of the azurophilic granules. Western blot analysis of the downstream effector molecule of MTOR, S6 kinase, showed increased phosphorylation of the enzyme upon ANCA treatment which was diminished in the presence of the PI3Kγ inhibitor, indicating that PI3Kγ acts upstream of MTOR and S6 kinase to promote exocytosis by ANCA. The ability of ANCA to modulate autophagy was next investigated with ANCA treated neutrophils displaying reduced LC3B-II lipidation compared with un-stimulated and normal IgG treated control cells. The work here indicates the importance of the gamma isoform of PI3K in auto-antibody induced neutrophil activation and highlights the potential therapeutic value of PI3K isoform selective inhibitors.

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Increased expression of TLR-2 and 4 in monocytes of obese individuals: association with the induction and progression of insulin resistance

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Obesity-associated chronic low-grade tissue inflammation is an important factor in the development of obesity-related pathologies, such as insulin resistance and type II diabetes. The cause and stimulus of persistent inflammatory activation in obesity is largely unknown. Toll like receptors (TLRs) are pattern recognition receptors expressed abundantly on monocytes and macrophages. TLRs and their activation lead to the increased transcription of pro-inflammatory cytokines, chemokines, and reactive oxygen species which may aggravate pathology in obese individuals. We investigated here whether TLRs could contribute to the progression and induction of diabetes in obese individuals. Peripheral blood and abdominal subcutaneous adipose tissue samples were collected from healthy as well as overweight and obese individuals, with or without diabetes. The expression of TLR2 and TLR4 was quantified by Immunohistochemistry, Flow Cytometry and RT-PCR. Proinflammatory cytokines were quantified by ELISA. Obese and overweight individuals showed significantly increased expression of TLR2 and TLR4 in monocytes and adipose tissue as compared with lean individuals ($P < 0.05$). Interestingly, a remarkably higher expression of TLRs in obese and overweight individuals with diabetes type II ($P < 0.05$) was observed. An increased expression of TLR2 and TLR4 was correlated with BMI ($P < 0.05$) but there was no major difference of TLR5 expression between lean and overweight/obese individuals. Moreover, a notable association of TLRs with the blood glucose level was observed ($P < 0.05$). Our findings suggest that the elevated expression of TLR-2 and 4, and associated-cytokines in overweight/obese individuals may play a role in obesity-associated inflammation and insulin resistance.

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Reduced glucose availability lowers MICA expression: a possible mechanism for tumour immune-evasion

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Natural killer group 2D (NKG2D) is an important activating receptor on the surface of natural killer cells and cytotoxic T-lymphocytes. MICA is the best characterized ligand for NKG2D and is up-regulated in various pathophysiological contexts, notably in cancer and viral infection. In cancer cells glucose metabolism is often abnormal (the Warburg effect) and glucose levels can be low in aberrantly vascularized tumours. We hypothesized that low glucose availability for metabolism could downregulate cell surface NKG2D ligand expression and thus lower the immunogenicity of malignant cells. To explore the impact of extracellular glucose on the expression of the NKG2D ligand, MICA, we cultured malignant cells in different glucose concentrations and found a positive correlation between glucose level and cell surface expression of MICA. Glucose concentration also correlated with cellular proliferation as measured using a CFSE-based flow cytometric assay. Real-time quantitative RT-PCR demonstrated a rise in MICA mRNA transcript levels with rising glucose concentration. A reduction in glucose concentration decreased MICA levels in a range of cell lines and reduced the lysis of these cells by NK cells in chromium-release killing assays. The observed reduction in killing promoted by a reduction in glucose level was mediated by the NKG2D-MICA interaction as demonstrated by a reduction in this effect using blocking anti-NKG2D antibody. These changes in MICA expression in response to low glucose concentrations may represent a novel immune-evasion mechanism in poorly vascularized, low glucose tumour environments. Elucidation of the downstream signaling intermediates could lead to the development of novel therapeutic approaches.

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UV upregulates NKG2D ligands expression by inducing mRNA stabilization via the EGFR pathway

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Ligands for the activating NKG2D receptor expressed by several T cell subsets and NK cells, such as MICA/B and ULBPs in humans, are known to be upregulated by several pathogens and various sterile stresses such as UV irradiation, heat shock, DNA damage and transformation. Upregulation by UV has mostly been attributed to the activation of the DNA damage repair (DDR) pathway. However, our current results show that mild UV doses trigger the Epidermal Growth Factor Receptor (EGFR), leading to MICA and ULBP2 upregulation through stabilization of their mRNA. This was independent of the DDR and increased cell surface expression of the ligands sufficiently to increase NKG2D-dependent cytotoxic responses by NK and gamma delta T cells. A/U rich element (ARE) sequences were identified in the 3′ untranslated region of MICA/B and ULBPs mRNA, and their involvement in mRNA stabilization by UV and EGF confirmed by their linkage to GFP cDNA. Our results provide a new insight in the mechanisms regulating stress-induced ligand expression. Moreover, they suggest that upregulation of NKG2D ligands by the EGFR pathway could be a common mechanism by which epithelial cell stress is relayed to the immune system. In this regard, the EGFR pathway is hyperactivated in many tumours and is triggered by several pathogens.

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Human native lactoferrin stimulates tumour necrosis factor alpha production via interaction with surface nucleolin

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Lactoferrin (LF) is an 80 kDa iron-binding protein that occurs naturally in most mammalian secretions (including tears, saliva and milk). The protein has been known for decades and has well characterised antimicrobial properties, including lipopolysaccharide (LPS) sequestration. More recently it had been proposed that LF also has immunomodulatory potential, with examples of both inhibitory and stimulatory effects recorded.

The current investigations have examined the ability of various forms of LF to impact on cytokine [tumour necrosis factor (TNF) α] production by THP-1 cells differentiated into macrophages by treatment with phorbol 12-myristate 13-acetate for 72 h. Cells were cultured for further 7 h with various concentrations of native LF from human or bovine milk (hLF and bLF), or with recombinant forms of hLF produced in either rice or *Aspergillus*. The role of LPS sequestration was determined by co-incubation with polymyxin B (PMB) and heat inactivation (1 h at 56°C).

The production of TNF-α by THP-1 macrophages was markedly upregulated in the presence of >10 μg/ml of native hLF or bLF. Heat treatment largely abrogated the effect, whereas PMB did not impact on cytokine production. In contrast, recombinant forms of hLF with identical amino acid sequences, but with different glycosylation profiles to the native material failed to induce vigorous TNF-α expression. Furthermore, co-culture with anti-nucleolin antibody (a putative membrane receptor for milk hLF) markedly inhibited TNF-α production induced by hLF.

In conclusion, the immunostimulatory effect of hLF in this system is likely mediated via surface nucleolin, is LPS-independent, and apparently relies on mammalian glycan chain expression.

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The role of IL-15 in the innate immune response to rhinovirus infections

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Rhinoviruses (RV) are the most common pathogen associated with acute asthma exacerbations, which are a major cause of morbidity. Deficient interferon (IFN) production has been reported in asthma during experimental RV infection, and this correlated with deficient IL-15 production. IL-15 is a key regulator of NK cells, though little is known about IL-15 and the NK cell response during RV infection. In this study, we used mouse models of RV-1B infection to investigate the role of type I IFN for IL-15 expression and NK cell activation in response to RV infection. Using a *BALB/c* RV-1B infection model, we found that IL-15 is induced at day 1 post-infection, which was followed by an influx into the airways of activated ($CD69^+$), $GranzymeB^+$ and IFN-$gamma^+$ NK cells at days 1−2 and into lung tissue at days 2−4 post-infection. Administration of an IL-15 neutralising antibody at the time of infection blocked the RV-induced NK cell response, demonstrating that IL-15 signalling is important. To investigate the role of type I IFN signalling, we infected type I IFN receptor knockout (IFNAR1ko) mice with RV-1B. These mice had deficient induction of IL-15, deficient activated NK cell responses and associated increased viral load and inflammation compared to wild type controls. These data provide evidence that RV-induced IL-15 and NK cell responses are dependant on type I IFN signalling. This is the first study to link type I IFNs, IL-15 and NK cells in the immune response to RV *in vivo*.

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M1 and M2 macrophages display profoundly different metabolic profiles and IL-10 production under hypoxic conditions

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Background: Macrophages have a wide range of immunological and non-immunological functions, ranging from clearance of apoptotic cells, tissue remodelling, and release of pro and anti-inflammatory mediators at sites of tissue damage or infection. Subsets of phenotypically distinct macrophages may be uniquely adapted to perform these roles. Phenotypically and functionally distinguishable monocyte-derived cell lines also express unique metabolic profiles, suggesting that metabolism may have the potential to regulate function.
Methods: In order to determine whether differentiated macrophages display similar profound metabolic differences, and whether these differences affect function, we differentiated primary human blood monocytes under a range of oxygenation conditions and assessed their metabolic fingerprints using NMR spectroscopy.
Results: Significant differences were seen in the metabolic profiles of M1 versus. M2 macrophages undergoing differentiation, with M1s displaying much reduced lactate levels, and corresponding increases in glucose, suggestive of gluconeogenesis via putative PFKFB3 (fructose-1,6-bisphosphatase) activity. M1s were demonstrated to be constitutively active under oxygen reperfusion conditions, with no corresponding metabolic changes following LPS stimulation. M2s, in contrast, showed an expected hypoxia profile of increased lactate levels under differentiation, and remained inactive in reperfusion conditions. However, production of IL-10 following LPS stimulation was shown to be significantly reduced in hypoxic conditions.
Conclusion: A model of permissive inflammation during M1 infiltration under hypoxia may be suggested. In normal tissues reperfusion and reduced recruitment may therefore drive resolution aberrant recruitment of M1 macrophages or persitent tissue hypoxia may result in chronic inflammation such as that seen in the rheumatoid synovium.

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Generation and phenotypic analysis of murine beta defensin 14 knockout mice

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Defensins are thought to be play a role in the protection against infection and immunological homeostasis, however their role is not completely characterised. Experimental induced mutations of defensin genes may enhance our understanding of their microcidal and immunological activity. We have generated mice that lack murine beta defensin 14 (mBD14), which was recently identified as the orthologue of human beta defensin 3 (HBD3). As the defensin genes are in close proximity to one another, we used oligonucleotide-mediated gene modification (oligo targeting) which created a specific mutation by inserting a 4 bp sequence in the *Defb14* gene creating a stop codon and a frameshift. The mutation has been confirmed in the homozygous mice at the genomic and RNA level.

HBD3 has been associated with ulcerative colitis, as patients with ulcerative colitis exhibit increased expression of HBD3 mRNA. Therefore the ability of Defb14 to maintain the immunological homeostasis of the gut, will be investigated by DSS induced colitis.

Further defensin genes (*Defb18* and *Defb48*) will be inactivated by oligo targeting, to create single and double homozygous mutants. These mutants will be phenotyped with the same methods used to analyse the mBD14 mutant mice. These experiments may help clarify the role of defensins in immunological homeostasis.

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Human complement factor H demonstrates binding to divalent metal ion-dependent and independent sites on *M. bovis* BCG and *M. smegmatis*

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Bacteria and other microorganisms have evolved many mechanisms for evading the host immune system. Some have developed the ability to down-regulate complement activation by binding complement down-regulatory proteins such as factor H (FH). Mycobacteria causing tuberculosis evade the immune system by actively gaining entry into phagocytes, and surviving intracellularly, but before cell entry, they are exposed to complement proteins. Previous work showed that *M. bovis* BCG binds purified FH (M. V. Carroll *et al*., 2009, *Mol Immunol* 46: 3367–3378). Binding was done in the absence of divalent metal ions (M^{++}), as nearly all reported FH binding to microorganisms is independent of M^{++}. Reinvestigation of FH binding surprisingly showed that FH binding to *M. bovis* BCG is much higher (five-fold) in the presence of either or both Ca^{2+} or Mg^{2+}. FH binding to *M. smegmatis* was also shown and has similar characteristics. When serum was used as a source of FH, FH demonstrate binding to both M^{++}-dependent and independent sites. No other serum protein competes for M^{++} -independent sites, but there is limited competition for the M^{++} -dependent sites by other serum proteins. Preliminary affinity chromatography tests suggest the M^{++} -dependent FH ligand is not a protein. Binding in the absence of M^{++} was optimal at physiological salt but M^{++} binding was optimal at low salt while both binding conditions had low pH optima of 5–6.

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Listeria monocytogenes induces mast cell degranulation which is independent of mast cell infection

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Listeria monocytogenes is a facultative intracellular pathogen which infects both phagocytic and non-phagocytic cells. Mast cells are known to be important during infection *in vivo*, since there is an increase in viable bacteria in the tissues of mast cell depleted mice. The interactions and responses of mast cells and *Listeria*, however, have not been fully investigated. Using bone marrow derived mast cells, the infection of and interaction with *Listeria* were examined *in vitro.*

Mast cells degranulate in response to *Listeria* and the bacterial toxin listeriolysin is required for this response. The kinetics, however, are slower than the degranulation in response to IgE and antigen. *Listeria* can infect and survive within mast cells, and viable bacteria can be seen 24 h after infection. This ability to infect mast cells, however, is independent of lysteriolyin and mast cell degranulation. Using fluorescent microscopy, future work will aim to understand the fate of *Listeria* during mast cell infection and how this affects mast cell biology.

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Human respiratory syncytial virus infection *in vivo* and *in vitro* induces airway epithelial cell expression of the B cell differentiation factor BAFF

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Background: In RSV disease, innate immune mediators expressed by infected airway epithelial cells are known to strongly influence both early inflammatory responses and the subsequent development of an adaptive immune response. The B lymphocyte differentiation factor, B cell activating factor of the TNF family, BAFF or TNFSF13B, has been shown to be present in autopsy samples from RSV Infected infants (Reed, J. L. *et al.* JID; 199:1128–38)

Aims: To determine if BAFF expression is elevated in the airways of infants with severe RSV Bronchiolitis and how RSV infection of primary Airway epithelial cells *in vitro* induces BAFF expression.

Methods and Results: BAFF protein was elevated in bronchiolar alveolar lavage fluid collected from the lungs of infants with severe RSV infection. Non infected control group infants (elective surgery) had lower levels ($P < 0.027$). To confirm these results BAFF mRNA was measured by taqman real-time PCR in bronchial brushings from patients and healthy infants. Average BAFF mRNA expression was 20-fold higher in samples obtained from infected infants ($P < 0.01$). In *In vitro* cultures of primary airway epithelial cells, infected with RSV A2, BAFF mRNA expression was induced 200-fold with maximum expression at 12 h post infection ($P < 0.01$) in an interferon beta dependant manner. Protein analysis showed expression of BAFF protein at 48 h post infection.

Conclusions: RSV infection of Airway Epithelial cells, *in vivo* and *in vitro,* induces expression of the B cell differentiation factor BAFF. This could drive B cell differentiation and Ab production in the infected lung.

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Maturation of equine monocyte-derived dendritic cells is not linked to CD83 expression

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Compared to human and mouse equine dendritic cells are not well characterized. Monocytes (Mo) were differentiated with equine GM-CSF and IL-4 generating monocyte-derived dendritic cells (MoDC). Time course experiments on iDC differentiation showed high expression of CD206 in early iDC with cells gradually becoming double positive for CD206 and CD83 and then loosing CD206 expression, reflecting previous findings. Functional attributes of iDC included high endocytic and phagocytic activity, low T cell activation potential and a lower ability to present antigen to autologous T cells. Activation with a combination of pro-inflammatory and anti-inflammatory cytokines conferred the best phenotypic transition to mDC. Reverse functional characteristics were observed in mDC compared to iDC.

In order to determine the expression of a broad range of markers for which no mAbs are available in the equine system, microarray experiments were performed to analyse the gene expression profiles between Mo, iDC and mDC. Those revealed the upregulation of the co-stimulatory B7 family ligands, PD-L1/PD-L2, ICOS-L and B7H3, in iDC. The chemokine receptor CCR7 was induced on iDC and further upregulated on mDC. Molecules that were reported to be involved in the regulation of CCR7-dependent chemotaxis, such as p38, JNK and ERK1/2, and CCR7-dependent migration, such as Rho and Pyk2 were differentially expressed. Chemokines detected that are involved in the migration of DC included CCL17, CCL19, CCL26, CXCL11, CXCL13 and CXCL6. In summary, this study demonstrates that equine iMoDC and mMoDC can be distinguished while CD83 is not a maturation marker as in the human system.

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Immunoglobulin like transcript 7 (ILT7)-mediated regulation of HIV-induced plasmacytoid dendritic cell (pDC) activation

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Immunoglobulin like transcript (ILT) 7 is a surface molecule selectively expressed by human pDC. ILT7 cross-linking suppresses plasmacytoid dendritic cells (pDC) activation and type I interferon (IFN-I) secretion following Toll-like receptors (TLR) 7/9 engagement. The bone marrow stromal cell antigen 2 (BST2, tetherin) is expressed by different cell types upon exposure to IFN-I and has been recently identified as a natural ligand for ILT7.

We investigated BST2/ILT7 expression in peripheral blood leukocytes (PBL) following HIV- or TLR7/9 ligand (TLR7/9L)-mediated pDC activation, and tested whether HIV-induced pDC activation is modulated by ILT7 cross-linking. Our data confirmed that ILT7 expression is specific to pDC, however the frequency of ILT7-positive pDC halved within 6 h of *in vitro* culture, a kinetic which was only modestly modified by HIV or TLR7/9L. BST2 expression was not affected by *in vitro* culture and was highest in monocytes, myeloid DC and B cells compared to pDC and T cells. BST2 upregulation in response to HIV-1 and TLR7/9L was observed only in pDC and monocyte and peaked in pDC at intermediate stimuli concentration, being only modestly increased at maximum stimuli concentration. This upregulation profile correlated with that of CD83 on pDC and IFN-alpha secretion. Finally, ILT7 cross-linking using a specific Ab inhibited IFN-alpha production in HIV and TLR7/9L-exposed PBL.

Our data suggests that pDC over-stimulation results in hyporesponsiveness or altered kinetics of pDC responses, and points at the BST2/ILT7 system as a regulator of pDC activation in response to HIV.

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Molecular analysis of the NF-kB pathway in regulatory dendritic cells

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Tumor escape in cancer patients is a complex process that involves the secretion of tumor derived soluble factors (TDSFs) and induction of a regulatory phenotype of various compartments of the immune system. The presence of TDSFs cause the accumulation of a heterogeneous population of cells called myeloid derived suppressor cells (MDSCs). These cells prevent the adequate maturation of antigen presenting cells (APCs) and render them incapable of processing and presenting antigens, thus, leading to tumor progression. We have previously shown that there exists a high number of MDSCs in cancer patients characterised by monocytes with an extremely low HLA-DR expression. These HLA-DRlow monocytes also express an unusually low amount of TLR-3, a phenotype that is similar to the MDSC model we are studying. This model is the recently discovered tolerogenic dendritic cell (DC) population called LILRB-1 DCs. These cells are an immature population of DCs that express a high number of NF-kB and IRF3 inhibitors, namely TNFAIP3 induced protein 1 (TNIP1), TNFAIP3 induced protein 3 (TNIP3) and novel protein NLRX1. We are currently studying the role that TNIP1 and TNIP3 play by down regulating their expression using siRNA. This down regulation could promote the translocation of NF-kB into the nucleus and hence give back these MDSCs their capacity to process and present antigens, thereby, mounting a strong anti-tumor immune response.

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IL-6 control of anti-microbial immunity through regulation of prostaglandin E2

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Transition from innate to acquired immunity is a critical checkpoint in the resolution of acute inflammation. This is defined by an initial influx of neutrophils, which are cleared and replaced by a more sustained mononuclear influx. We have shown that IL-6 acting through its soluble receptor (sIL-6R) is instrumental in directing this response through activation of non-hematopoietic stromal cells. We now propose that supplementation of this IL-6-directed outcome would improve anti-microbial host defence and enable bacterial clearance. Using models of acute resolving bacterial peritonitis, we show that IL-6-deficient mice display impaired clearance of peritoneal *Staphylococcus epidermidis*. This inability to handle infection is associated with increased bacteremia, suggesting that IL-6 restricts dissemination into surrounding tissues and organs. This response was not attributable to an inherent defect in innate neutrophil effector function, but is likely arise through a loss of local anti-microbial host defence. Local administration (i.p.) of an IL-6-sIL-6R fusion protein (HDS) dose-dependently (10−1000 ng/mouse) improved bacterial clearance and reduced bacterial dissemination. To identify potential mechanisms for IL-6/sIL-6R involvement, we used Q-PCR to examine IL-6 regulation of enzyme systems linked with anti-microbial defence. In human peritoneal mesothelial cells (HPMC), HDS was found to induce cyclooxygenase-2 (COX-2), and was associated with the generation of prostaglandin-E2 (PGE2) as quantified by mass spectrometry. Evaluation of neutrophil function showed that PGE2 suppresses neutrophil activity. We propose that IL-6 regulation of PGE2 may serve to trigger neutrophil clearance and the removal of bacteria. This hypothesis is currently being chased in our models of acute bacterial peritonitis.

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Identification of the molecular hotspot in the α2 helix of CD1d that is responsible for species differences in iNKT cell receptor binding

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Background: Studies in mice show that iNKT modulation is a promising therapeutic avenue for autoimmunity, cancer and infection. However, translation of these studies into clinical trials has failed, indicating important species differences in the mouse and human iNKT/CD1d systems. We have recently shown that human iNKT activation in response to self-lipids depends on the structure of the iNKT-TCR's CDR3b loop. The structure of the iNKT-TCR:CD1d interaction shows that the likely target for this protein-protein interaction is the α2 helix of human CD1d (hCD1d), which is poorly conserved with mouse CD1d (mCD1d). We therefore hypothesised that a key human−mouse species difference may be that hCD1d and mCD1d differ in their ability to support this interaction.
Methods: Using lentiviral technology we generated a series of stable T2-lymphoblast lines expressing either wild-type hCD1d or mCD1d, or partially and fully 'humanised' mCD1d or 'mousified' hCD1d molecules. The binding of human high-affinity iNKT-TCRs to these CD1d variants was measured using fluorescent iNKT-TCR-tetramers generated by *in vitro* refolding.
Results: All CD1d molecules supported binding of human iNKT-TCRs when loaded with the strong antigen KRN7000. Conversely, hCD1d, but not mCD1d, supported autoreactive binding of these iNKT-TCRs. These differences were caused by a short sequence within the α2 helix of CD1d.
Conclusions: The insights obtained from this study form the basis for rational design of a humanised CD1d mouse model which will enable us to conduct cross-disciplinary studies on the selection and function of iNKT cells *in vivo*, with enhanced potential for successful translation into human clinical trials.

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Dissecting the response of dendritic cell subsets to the parasitic helminth *Schistosoma mansoni*

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In contrast to Th1 related bacteria, viruses and protozoa, relatively little is known about how dendritic cells (DCs) become activated and function in response to Th2 associated parasitic helminths. Murine bone marrow cultured with Flt3-L differentiates into DC subsets thought to be representative of populations generated *in vivo* in the steady-state. Using FL-DCs we can assess how steady-state DCs respond to soluble egg Ag (SEA, a potent Th2-inducing antigen) from the medically important helminth *Schistosoma mansoni*. Phenotypic activation and cytokine production by FL-DCs exposed to SEA was compared to those stimulated with bacterial Ag. SEA-pulsed FL-DCs displayed low-level activation and released minimal amounts of the inflammatory cytokines IL-6, TNF and IL-12. Unexpectedly, FL-DCs responded to SEA exposure by secreting Type I Interferons. Although phenotypically similar to unstimulated DCs, SEA-conditioned FL-DCs capably induced antigen-specific Th2 responses following adoptive transfer into naïve mice, though less efficiently than SEA-pulsed GMCSF DCs. Ongoing work is addressing whether regulatory pathways, including IL-10 production and CD200 receptor/ligand interaction, may play a role in restricting the Th2 priming ability of FL-DCs, and assessing the influence of Type I IFN release on FL-DC activation and function in this Th2 setting. Further work comparing differences in activation and function of Flt3-L and GMCSF-generated cells is addressing whether there is a functional dichotomy between steady-state and inflammatory DC subsets *in vivo* in the context of Th2 pathogens, and whether *ex vivo* murine and human DCs respond similarly to SEA.

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A quantifiable proliferative burst of tissue macrophages restores homeostatic macrophage populations after acute inflammation

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Macrophage (MØ) biology is routinely modelled in the peritoneal cavity, a vascular tissue readily infiltrated by leukocytes during inflammation. After several decades of study, no consensus has emerged regarding the importance of in situ proliferation versus peripheral monocyte recruitment for the maintenance of tissue resident MØs. By applying specific measures of mitosis, we have monitored tissue MØ proliferation during newborn development, adulthood and acute resolving inflammation in young adult mice. Despite the vascular nature of the tissue and ease of peripheral leukocyte entry, tissue MØs in the newborn increase in number by local proliferation. On the contrary, in the adult, tissue MØ proliferation is considerably reduced and most likely provides homeostatic control of cell numbers. Importantly, during an acute inflammatory response, when substantial numbers of inflammatory MØs are recruited from the circulation, tissue-resident MØs survive and then undergo a transient and intense proliferative burst in situ to repopulate the tissue. Our data indicate that local proliferation is a general mechanism for the self sufficient renewal of tissue MØs during development and acute inflammation and not one restricted to non-vascular tissues, which has implications for the therapeutic modulation of MØ activity during the resolution of inflammation. Ongoing studies aim to delineate the underlying molecular mechanisms that control the proliferative potential of tissue MØs.

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CX_3CR1^{int} lamina propria cells accumulate during multiple models of colitis and can be distinguished from CX_3CR1^{hi} lamina propria cells by Ly6C expression

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The lamina propria (LP) is home to numerous small myeloid populations, particularly dendritic cells (DCs) and macrophages, which greatly influence immune homeostasis. The balance of these LP cell (LPC) subpopulations is altered during inflammation compared to the steady-state. The objective of this project is to further characterise LPC subsets, continuing from our previous description of E-cadherin$^+$ DCs. CX_3CR1-GFP knock-in reporter mice have allowed us to identify LPC subsets from steady-state and inflamed colonic tissue for further characterisation. Here we show that CX_3CR1^{int} LPCs increase in number and frequency in both the T cell transfer and *Helicobacter hepaticus* + anti-IL-10R models of colitis in mice. Our results suggest that CX_3CR1^{int} LPCs are inflammatory monocyte-derived and may overlap with E-cadherin$^+$ DCs. CX_3CR1^{int}, but not CX_3CR1^{hi}, LPCs from inflammatory settings express Ly6C by flow cytometry. This will therefore allow us to distinguish between these populations based on their expression of Ly6C without the need for the CX_3CR1-GFP reporter, enabling the future characterisation of these cells in all mouse strains.

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The impact of IL-4 on activation and function of dendritic cells: expression of the alternative activation product RELMα is required for optimal Th2 induction

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The archetypal Th2 cytokine IL-4 has potent effects on multiple cell types of both the innate and adaptive immune system. We have comprehensively characterised the impact of IL-4 on the alternative activation of *in vitro* derived dendritic cells (DC). In this work we expand on these *in vitro* findings by detailing the impact of both helminth driven and exogenously delivered IL-4 on the alternative activation of DC populations *in vivo*, at different tissue sites. In addition, we have been comparing the *in vitro* and *in vivo* priming capacity of wild type GM-CSF derived bone marrow DCs with those deficient in expression of either the IL-4 receptor or RELMα, a molecule associated with alternative activation. This work has revealed a previously unknown requirement for DC expression of RELMα in the induction of IL-10 and in the optimal induction of Th2 responses. Together, these data highlight the direct and dramatic influence of IL-4 on DCs both *in vitro* and *in vivo*.

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Epstein–Barr virus latency and weaker NK-cell responses in children – exploring the immune modulating potential of EBV

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Objective: Epstein–Barr virus (EBV) is a gamma-herpesvirus that is widely spread in human populations. Primary EBV infection often occurs during early childhood and is usually asymptomatic. After primary infection, EBV establishes latency. In order to persist, EBV uses various immune modulatory mechanisms but the effect of EBV latency on the innate immune system in children is largely unknown. Previously, we found that EBV seropositive 2-year old children had lower monocyte-induced NK-cell IFN-g responses. This raised the question whether herpesvirus infection could modulate immunity during early life. Here we aimed to further characterize the NK-cell response in EBV seropositive children, and also relate it to the timing of seroconversion.

Methods: PBMC from 5-year-old children in a cohort of known EBV serostatus were used for *in vitro* functional studies. Following stimulation with K562 cells, or IL-15 + peptidoglycan, NK cells were assayed for intracellular IFN-g and CD107a expression. Further, a range of cytokines was assayed in plasma.

Results and conclusions: EBV seropositive children had a weaker IFN-g and degranulation response to K562 cells. The timing of EBV seroconversion influenced results as early acquisition (before 2 years), versus late (after 2 years) was associated with the lowest NK-cell response. However, the early EBV converted children had the highest circulating levels of IFN-a, indicating that there was no general attenuation of anti-viral immunity. This could suggest that NK cells in EBV+ children are refractory to innate activation signals, possibly through EBV suppressive mechanisms that are maintained in latency.

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Does the inflammatory regulatory protein Mediterranean Fever (MEFV) play an important role in the bovine macrophage response to bacterial infection?

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Mutations in the human Mediterranean fever (MEFV) gene are associated with the autoinflammatory disease Familial Mediterranean Fever (FMF), which is characterized by recurring, spontaneous episodes of fever and localized inflammation. MEFV is composed of several domains, including an N-terminal Pyrin domain. Proteins containing Pyrin domains are frequently incorporated into inflammasomes; cytoplasmic multiprotein complexes mediating the proteolytic activation of caspase-1, thus regulating the release of active interleukin (IL) 1β and IL18. The autoinflammatory disease associated with MEFV mutations and the presence of the Pyrin domain suggest that MEFV plays an important role in the regulation of inflammation. We have recently discovered that MEFV is rapidly up-regulated in bovine macrophages after infection with *Salmonella enterica* serovar Typhimurium, *Mycobacterium bovis* and stimulation with *Escherichia coli*-derived lipopolysaccharide (LPS). Therefore, we hypothesize that MEFV plays an important role in the response of bovine macrophages to pathogens. RT-PCR analysis has revealed that there are two dominant splice variants expressed in bovine macrophages and the expression of additional splice variants is induced by LPS stimulation. The splice variants do not differ in their predicted amino acid sequences and therefore their functional importance is not understood. Similar to murine MEFV, the predicted bovine protein lacks the C-terminal Spry domain, where the majority of FMF associated mutations are located. We are currently furthering our understanding of bovine MEFV by extending our analysis of the splice variants, investigating sequence variation amongst bovine breeds and studying the effect of MEFV knockdown by siRNA on macrophage activation and inflammasome activity.

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RNA-seq based transcriptomic characterization of helminth-elicited alternatively activated macrophages provides insights into cellular effector functions

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Alternatively activated macrophages (AAM?), induced by Th2 promoting cytokines IL-4 and IL-13 are implicated in diverse disease settings including cancer, fibrosis, allergy and helminth infection. In spite of this, little is understood about the physiological roles of AAM?.

Using RNA-seq we have characterized the phenotype of AAM?. We profiled transcriptomes of WT and IL4Rα deficient peritoneal macrophages elicited by *Brugia malayi* (helminth) infection (AAM?) and compared these to thioglycollate recruited M?. Our analysis both consolidates our understanding of alternative activation and provides new unexpected insights into AAM? function and regulation. These findings include:

1 Oxidative metabolism, maintained by the transcription factor PPARγ, is described as critical for alternative activation. Our metabolic pathway analysis and *cis*-element analysis support the notion that alternative activation is maintained by PPAR transcription factors. PPARγ however is down-regulated in our model, and we propose that alternative activation in this setting is maintained via PPARδ. We are testing this hypothesis.

2 A systematic comparison of differentially expressed cytokines and chemokines, and their receptors, provides consistent evidence that AAM? contribute towards maintaining an eosinophil and B-cell rich environment via multiple redundant routes. Further AAM? exhibit limited migration potential and thus are unlikely to contribute to naïve T-cell activation.

3 Complement components comprise some of the most abundant AAM?-derived transcripts and are regulated in an IL4Rα-dependent manner. The role of complement in helminth infection is relatively unexplored. We provide evidence that a FicolinA-complement axis may be a key effector function of AAM?.

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Lung NKT cells modulate NK and T cell activity to drive Th2 responses during respiratory viral infection

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Natural Killer T (NKT) cells are innate cells that upon activation with glycolipids, rapidly secrete a diverse array of cytokines that shape innate and adaptive responses. Their ability to rapidly respond to stimulation makes them attractive therapeutic targets. Lung NKT cells have been the focus of intense study in asthma and allergy. Mouse models have demonstrated that lung NKT cells are essential for airway hyperreactivity, a cardinal feature of asthma. However these findings are controversial in humans, and how lung NKT cells affect responses to respiratory viral pathogens that exacerbate asthma and allergy remains unclear.

In this study, we have addressed the influence of lung NKT cells on immunity to Respiratory Synctial Virus (RSV). Activated lung NKT cells promote an early Th2 lung environment upon RSV challenge, ablating CD8 T cell recruitment while driving NK cells and eosinophilia. Crucially, they inhibit IFN-γ and promote IL-10 production by NK cells, and promote IL-4 production by T cells, suggesting these cells maintain the Th2 environment. NK cell depletion reduced pathology, ablated eosinophilia (by reducing eotaxin-2 and increasing IFN-γ levels) and inhibited lymphocyte recruitment to the airways, confirming their importance. RSV-specific memory is Th2-biased after lung NKT cell activation, and upon rechallenge promotes a Th2-driven pulmonary eosinophilia, and RSV-specific serum IgE, in the absence of significant pathology.

This study highlights novel mechanisms by which NKT-cell-driven Th2 responses are maintained in the lung. These mechanisms can be targeted therapeutically not only in viral disease models but also in models of asthma and allergy.

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Effect of T follicular helper cells on regulation of mucosal immunity to upper respiratory tract pathogens by novel immunological adjuvants

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Background: Stimulation of the innate immune system is known to have an important role in the initiation and regulation of adaptive immunity. Therefore, inclusion of some immunological adjuvants such as Toll-like receptor ligands, which trigger early innate responses to enhance the adaptive responses, is crucial to vaccine effectiveness. T follicular helper cells (TfH) have recently been shown to be crucial in germinal centre function and in regulation of adaptive immunity. The aim of the study is to investigate whether and how TLR ligands regulate T and B cell immunity to some respiratory tract pathogens through TfH cells.
Methods: Peripheral blood, nasopharyngeal swab and adenotonsillar tissues are collected form children and adults undergoing adenotonsillectomy. T cell proliferation and B cell antibody production analysed by CFSE and ELISA respectively. Effect of TLR ligands on TfH cells and their function are analysed by flowcytometry and intracellular cytokine staining, after stimulation of adenotonsillar cells with TLR ligands. Kinetics of antibody and cytokine production will also be analysed by ELISA.
Results and conclusion: Results suggest that TLR-9 ligand CpG-DNA can significantly enhance the antibody responses to pneumococcal protein (choline-binding protein A) in adenotonsillar cells. CpG-DNA appears to increase the proportion of TfH cells in adenotonsillar cells, which could be inhibited by the specific TLR inhibitor. However, TLR-2 ligand (BLP) seems to downregulate the proportion of TfH cells. Understanding the mechanisms by which TLR ligands regulate adaptive immunity through TfH cells may lead to a successful vaccination strategy against respiratory infections.

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Stage-specific Rab GTPase function in phagosomes containing *Candida albicans*: tools to resolve the molecular mechanisms of pathogen-phagocyte interplay

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Phagosomes mature by fusion and fission with endocytic and lysosomal compartments, during which they acquire degradative properties; a process regulated by the well characterised Rab GTPases, Rab5 and Rab7. Several pathogens have evolved mechanisms to subvert phagosome maturation by manipulating Rab function. The clinically important fungus, *Candida albicans*, is able to escape macrophages by producing hyphal filaments. Combining a *C. albicans* phagocytosis model with sophisticated live video microscopy, we studied Rab GTPase activity in maturing phagosomes using siRNA and GFP-/RFP-tagged variants of native and mutant Rabs. As expected, siRNA-mediated knockdown of Rab7 in macrophages blocked phagosome maturation as demonstrated by reduced acidification of phagosomes containing yeast cells. In addition we are studying Rabs with poorly defined phagosomal function (Rab2, 9, 10, 11, 14, 18, 22a, 23 and 35) and these were visualised during live cell phagocytosis to determine their sequence of function in the context of the Candida phagosome. For example, confocal and live microscopy demonstrates localization of GFP-Rab14 to phagosomes that contain *C. albicans*. Interestingly, Rab14 is known to actively block phagosome maturation upon Mycobacterium phagocytosis, thus favouring pathogen survival. However, Rab14 siRNA knockdown was associated with a five-fold increase in macrophage lysis by hyphae, which was not attributed to altered uptake of *C. albicans*. These results suggest that Rab14 promotes, rather than blocks phagosome maturation following uptake of *C. albicans*. This approach provides mechanistic insight into the molecular processes driving phagosome maturation at the pathogen-phagocyte interface and may identify novel targets for therapeutic intervention.

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Defective anti-bacterial immunity in the allergic lung

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Asthma and bacterial pneumonias are major causes of human mortality and morbidity throughout the world. To date many studies have investigated the possibility that bacteria exacerbate asthma but only a handful considers that asthma may cause a bacterial exacerbation. Recent evidence suggests that bacterial infections cause serious complications in patients with asthma and that asthmatics show a two-fold increased risk of invasive pneumococcal disease. We show for the first time, using a murine model of asthma, that mice with house dust mite induced allergic airways disease have increased susceptibility to *Streptococcus pneumoniae* infection. Furthermore, the molecular pathways leading to the production of neutrophil chemoattactants in the lung are compromised and that despite the complexity of anti-bacterial pathways that are disrupted, the re-introduction of a single chemokine to mice with allergic airway disease enables clearance of *S. pneumoniae* that would otherwise prove fatal. These findings highlight the role for specific innate immune pathways on the asthmatic lung that participate in susceptibility to bacterial pneumonia.

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Adenovirus vector delivery promotes natural killer cell recognition by stimulating the expression of multiple activating ligands

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Adenovirus (Ad) is the most commonly used vector in Gene Therapy. Replication-deficient Ad vectors provide the most efficient technology available for *in vivo* gene delivery, yet their utility can be limited by their recognised proinflammatory properties. A full appreciation of the molecular mechanisms driving this immune stimulation is required to optimise vectors for their application in human gene therapy protocols and as a vaccine carrier. Our previous studies have shown that human NK cells are capable of recognising Ad vector transduced cells. We therefore sought to evaluate changes to the protein content of the plasma membrane following delivery of a first generation Ad vector. Cells were subject to stable isotope labeling with amino acids in cell culture (SILAC), cell surface glycoproteins fractionationed, and then analysed by mass spectroscopy. This enabled a comparison of >600 cell surface proteins. Ad vector delivery was associated with the upregulation of a substantial subset of host cell proteins (>100), data will be presented. Crucially, MICA, a ligand for the ubiquitously-expressed NK cell receptors NKG2D exhibited the largest increase following vector delivery. More detailed analysis revealed that vector delivery stimulated the expression of multiple NK cell activating ligands (notably also MICB, ULBP2, CD155) to render Ad vector transduced cells vulnerable NK cell-mediated cytolysis. The stimulation of MICA and MICB could be eliminated by deletion of the E4 gene region.

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Probing the function of PILAR/KACL, a novel C type lectin

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Background: The human Natural Killer Gene Complex (NKC) on chromosome 12 encodes proteins in the KLRB and the CLEC2 families. These proteins include several receptor-ligand pairs where the receptor and ligand share close genetic linkage and similar signaling motifs, notably CD161/LLT1 and NKp65/PILAR (or KACL). Crosslinking CD161 on NK cells inhibits cytotoxicity and cytokine secretion, although its function on T cells is unclear. PILAR/KACL is reported as a ligand of CD161, although the level of binding has been controversial and to date its function *in vivo* is still unclear.
Aim: We aimed to generate soluble molecules to define the expression and binding of PILAR/KACL on lymphocytes and other tissues *in vivo* and hence dissect out its specific function.
Methods: We generated recombinant PILAR/KACL using two independent methods, one in monomeric form and a second using recombinant Fc fusion molecules. The functional impact of soluble PILAR/KACL interactions with CD161 on T cells was assessed using sorted T cells and intracellular cytokine staining after exposure to bead- or plate-bound soluble ligand.
Results: Soluble PILAR/KACL did not show substantial binding to $CD161^+$ lymphocytes. Consistent with this we could not observe specific modulation of $CD161^+$ T cell function after exposure to PILAR/KACL. Unexpectedly, PILAR protein specifically bound a subset of B cells in healthy donors.
Conclusions: Our combined data suggest that binding of PILAR/KACL to CD161 is limited and does not have a substantial impact on T cell triggering. Binding of PILAR/KACL to B cells suggests a novel potential biologic function *in vivo*.

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Analysis of macrophage migration towards and engulfment of *Candida albicans* using sophisticated live cell video microscopy

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Candida albicans is an opportunistic fungal pathogen that can cause life-threatening systemic infections in immunocompromised hosts. Phagocytosis of *C. albicans* by cells of the innate immune system is an essential component of the immune response to infection. We show here for the first time a detailed minute by minute account of the specific effects of *C. albicans* viability, cell wall composition, morphogenesis and spatial orientation on two distinct stages (macrophage migration and engulfment of bound *C. albicans*) of the phagocytosis process.

Analysis of macrophage paths towards *C. albicans* using sophisticated tracking software revealed that the speed of macrophage migration was dependent on the glycosylation status of the fungal cell wall, but not on cell viability or morphogenic switching from yeast to hyphal forms.

Macrophages rapidly engulfed viable and UV-killed *C. albicans*, but the rate of engulfment was significantly slower for all glycosylation and yeast-locked morphogenetic mutants examined. Hyphal cells were engulfed at a slower rate than yeast cells, especially those with hyphae in excess of 20 μm, but there was no correlation between hyphal length and the rate of engulfment below this threshold. We show that spatial orientation of the hypha was another important determinant of the rate of engulfment.

This study reveals unique insight into the complex mechanisms that govern *C. albicans* phagocytosis by macrophages and could serve as a blueprint for the study of host interactions with other pathogens and dying cells.

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Next generation sequencing reveals strain sensitivity to *Mycobacterium bovis* in bovine macrophages

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Introduction: Bovine infection with *Mycobacterium bovis* is an important agricultural problem in the UK and poses a wildlife risk in many countries. An early macrophage response is essential for infection control. Using RNA-Seq we examined the transcriptomic response to two strains of *M. bovis* in bovine macrophages to illuminate innate mechanisms in early infection.
Methods: Monocyte-derived macrophages from six female Holstein-Friesian cattle were infected with *M. bovis* strains AF2122/97 or G18 at a MOI of 5:1 or cultured without infection. RNA was isolated at 2, 6, 24 and 48 h and sequenced using the Illumina GAII. Count data was assigned at the gene level with paired sample analysis used to assess differential expression using a FDR cutoff of 1%.
Results: Examination of the response at 2 h (infection versus uninfected control) revealed that AF infection regulated more genes than G18 (240 versus 195). Forty-five percent of regulated genes overlapped both infections. Gene Ontology analysis revealed strong enrichment for innate immune biological processes and pathways in regulated genes. This persisted over time despite an amelioration of the transcriptomic response from 6 h onwards. In both infections CXCL5 was highly responsive and, along with CCL5, IL-6 and IL-8 was amongst the genes remaining regulated throughout the time-course.
Conclusions: The bovine macrophage transcriptomic response to infection with mycobacteria is rapid and strain sensitive. Gene expression rises between 2 and 6 h; both numbers of genes and degree of fold change decline thereafter but innate immune signatures remain strongly represented in regulated genes.

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Structural flexibility and ligand-binding characteristics of the macrophage dengue virus receptor CLEC5A

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CLEC5A is an important receptor present on the surface of macrophages and has been shown to be a critical receptor for dengue virus. The binding of dengue virus to CLEC5A triggers signaling through the associated adapter molecule DAP12, stimulating proinflammatory cytokine release. We have crystallized an informative ensemble of CLEC5A structural conformers at 1.9 Å resolution, which demonstrates how an on-off extension to a β-sheet acts as a binary switch regulating the flexibility of the molecule. This structural information combined with molecular dynamics simulations suggests a mechanism whereby extracellular events are transmitted through the membrane and influence signaling via DAP12. Using BRET, we have shown that CLEC5A is homodimeric at the cell surface. Recombinant CLEC5A binds to dengue virus serotypes 1–4. We used blotting experiments, surface analyses, glycan microarray and docking studies to investigate the ligand binding potential of CLEC5A with particular respect to dengue virus. CLEC5A does not have the structural characteristics of typical sugar-binding C-type lectins and recombinant CLEC5A does not bind to any of a diverse array of carbohydrates. This study provides a rational foundation for understanding the dengue virus-macrophage interaction, and the role of the interaction with CLEC5A in dengue virus-induced disease.

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Mechanisms of the lymphocyte hypo-responsiveness observed after multiple infections of the skin with *S. mansoni* cercariae

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Schistosomiasis is an important helminth disease affecting 200 million people. In endemic areas repeated exposure to the Schistosome cercariae occurs throughout life. To mimic this, a multiple-infection model has been developed in the lab, to specifically investigate the early immune responses in the skin and determine how this differs from a single infection. Using this multi-infection model, previous experiments have shown that multiple exposures of the skin to *S. mansoni* cercariae results in the development of lymphocyte hypo-responsiveness. This project aims to understand these early immune events that lead to the observed lymphocyte hypo-responsiveness, prior to maturation of the cercariae. In particular the expression and function of regulatory markers in both the dermal cells and the skin draining lymph nodes during multiple (4×) infection as compared to a single (1×) infection have been investigated. So far, lymphocyte hypo-responsiveness has been confirmed *in vitro* with reduced cytokine production and proliferation observed in response to parasite antigen after multiple-infections. Also, in the skin draining lymph nodes after multiple infections, the expression of PD1 was increased on CD4+ T cells suggesting a state of anergy. In addition, the expression of PD1 ligands, PDL-1 and PDL-2, was increased on the macrophage populations. Differences in the proportions of FoxP3+ T regulatory cells and FoxP3+ macrophages were also investigated. The results shown give an insight into the mechanisms which give rise to the observed hypo-responsiveness and suggest possible therapeutic targets.

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Bovine macrophage expression of the *SIRPB* genes is altered by infection with *Theileria annulata* and activation with LPS

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The tick-borne protozoan parasite *Theileria annulata* infects bovine macrophages (mϕ), causing an overwhelming inflammatory response in Holstein (*Bos taurus*) cattle but not in Sahiwal (*Bos indicus*) cattle. Holstein and Sahiwal mϕ have distinct transcriptome profiles; in particular signal regulatory protein (SIRP) beta 1 is present at 24.5-fold higher levels in Holstein-derived mϕ. The SIRP family, which includes SIRPB1, SIRPB2 and SIRPB3, regulates inflammatory responses. A lysine residue in the transmembrane region of SIRPB1 interacts with DAP12, and may promote phagocytosis and pro-inflammatory cytokine secretion. We hypothesize that the difference in SIRP expression underlies the observed genetic tolerance to *T. annulata* infection. Therefore we are investigating the regulation of SIRP expression in bovine mϕ. Sequence analysis has revealed that the majority of Sahiwals do not express SIRPB1 and have SNPs in the promoter region of the gene. Potentially these may affect transcription factor binding and lead to the observed differential expression. In Sahiwal and Holstein-derived mϕ both expressing *SIRPB1* and infected with *T. annulata*, the predominant splice variant lacks the transmembrane encoding region, and therefore may not function. However increased expression of *SIRPB3,* which may increase cell activation, is seen in Holstein mϕ. Further investigation of expression in uninfected mϕ activated with LPS showed an increase in *SIRPB1* and a decrease in *SIRPB2* after 48 h. SIRPB3 expression did not change. Expression of the *SIRPB* genes is altered by both infection and activation, which may change the balance of activating and inhibitory signalling and determine the progression of infection.

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Critical roles for C5aR and C3aR in renal ischemia/reperfusion injury

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Both C5a and C3a (generated from complement activation) have broad pro-inflammatory potential, but their roles in renal ischemia/reperfusion (I/R) injury are not well-defined. In this study, we investigated the contribution of C5a receptor (C5aR) and C3a receptor (C3aR) to renal I/R injury. We show that double deficiency of the receptors for C3a and C5a (C3aR/C5aR) significantly protected mice from acute kidney injury, although C5aR or C3aR single deficiency also provide certain levels of protection; this was evident by the reduction of renal functional impairment and tubule destruction, and the increase of renal expressing kidney injury molecule (KIM-1). We also show that deficiency of C3aR/C5aR reduced cellular infiltration of $CD45^+$, $Gr\text{-}1^+$ and $F4/80^+$ cells and lowered expression of pro-inflammatory cytokines (i.e., TNF-a, IL-1β, IFN-γ) and chemokines (i.e. KC, MIP-1α, MIP-1β, MCP-1) in post-ischemic kidneys. Furthermore, experiments with bone morrow chimeras (between WT and receptor deficient mice) and primarily cultured renal tubular epithelial cells suggest that C3aR/C5aR expression on both renal and circulating cells contributes to the pathogenesis of renal IR injury. Therefore, our findings demonstrate that both C5aR and C3aR signaling contribute to the pathogenesis of renal I/R injury, which offers new insight into the mechanism by which complement mediates renal IR injury, and has relevance for therapeutic strategies.

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A novel role for C5a in NK cell functional regulation

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Natural killer (NK) cell dysfunction is associated with chronic inflammation or high inflammatory profile in certain pathologies. However, it is unclear if and how inflammatory mediator could contribute to NK cell dysfunction. In this study we show that anaphylatoxin C5a interacting with its receptor C5aR negatively regulates NK cell activation and function. Employing C5aR deficient mice and NK cell dependent tumour clearance model, we found that C5aR deficiency significantly enhances *in vivo* tumour elimination. In the absence of C5aR, NK cells exhibit a hyperactive phenotype, with a striking increase in their surface expression of NKp46 (a pivotal activation molecule of NK cells), and enhanced functional activities (i.e. tumour cell killing, expression of CD107a and IFN-γ/TNF-α production. Conversely, C5a stimulation down-regulates NKp46 expression and impairs NK cell effector functions, which compromises the changes in signalling transduction pathways (i.e. up-regulation of cAMP/PKA and inhibition of ERK). Our findings demonstrate a pivotal role for C5a in regulation of NK cell function, which enhances our understanding of NK cell functional modulation by inflammatory mediators. It may help to explain the recent observation that C5aR signalling has negative impact on the immune surveillance of cancer and have therapeutic implications of C5aR manipulation in human cancer.

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Intranasal responses to murine Nod1 ligand in BALB/c mice

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Nucleotide-binding oligomerisation domain (Nod)-like receptors (NLRs) are involved in sensing bacteria and stimulating innate responses during infection. Nod1 and Nod2 are NLRs that detect bacteria by recognising different components of bacterial peptidoglycan. Nod 1 recognises the peptidoglycan component of Gram-negative bacteria [γ-D-glutamyl-meso-diaminopimelic acid (meso-DAP)] and Nod2 recognises the peptidoglycan component of Gram-positive bacteria (muramyl dipeptide). Nod1 is present in the cytosol of pulmonary cells and is well-placed for the detection of intracellular pathogens. *Burkholderia pseudomallei* is an intracellular bacterium that causes the potentially fatal disease melioidosis in humans. This disease is endemic in tropical regions and has a serious outcome for many infected individuals. No vaccine is currently available, antibiotic treatment needs to be aggressive and relapse of the disease is frequent. Therefore, novel strategies for treating this disease are required. We aimed to determine the pulmonary and systemic immune response following intranasal delivery of the Nod1 ligand FK565 in BALB/c mice and the effect of FK565 on intranasal infection with *B. pseudomallei*. A significant increase in peripheral and pulmonary chemokines, KC and CCL2, was observed 6 h following i.n. dosing with FK565 in comparison to PBS controls. This was accompanied by an increase in neutrophils and NK cells in the lung. Intranasal dosing with FK565 had no significant effect on survival during intranasal challenge with *B. pseudomallei*. However, a significant trend in survival associated with time of FK565 dosing was observed suggesting further optimisation of dosing schedules may affect the progression of disease.

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Critical role for C5a in the pathogenesis of urinary tract infection

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Previous studies have shown that most human uropathogenic E. coli strains are resistant to complement mediated killing and complement activation is harmful instead of beneficial for the host in urinary tract infection (UTI). However, the mechanisms by which complement activation promotes the process of UTI are unclear. In this study we show that anaphylatoxin C5a generated during complement activation is an important pathogenic factor for UTI. Employing C5aR−/− mice and an ascending urinary tract infection mouse model, we found that, compared to WT mice, C5aR−/− mice exhibited a lower rate of kidney infection (3/12 versus 12/12), reduced bacterial load in the infected kidneys (0.5 versus 3.5 Log10 c.f.u) and bladder (2.9 versus 4.5 Log10 c.f.u), and reduced renal tissue damage. The use of C5aR antagonist (C5aRa) confirmed these data by showing that C5aRa treatment protected mice from kidney infection. To understand how C5a contributes to the pathogenesis of UTI, we determined the effects of C5a on renal tubule cell activation in response to *E. coli* by measuring pro-inflammatory cytokine production. We found that C5a stimulation significantly enhanced the production of TNF-α/IL-6/IL-1β by murine tubular epithelial cells in the presence of LPS or heat killed E. coli. Our findings demonstrate a critical role for C5a in the pathogenesis of ascending urinary tract infection and suggests an important mechanism, namely C5a acting in synergism with TLR4 to promote epithelial inflammation and damage. It also suggests the potential for therapeutic application of C5aRa in urinary tract infection.

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Ageing impairs the ability of macrophages to phagocytose myelin debris

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The immune system has beneficial roles in central nervous system repair and macrophages in particular are important in remyelination. Macrophages are potent phagocytic cells and are involved in clearance of myelin debris. It has been shown that myelin debris inhibits remyelination. Many studies report that macrophage function is altered as a result of ageing. Interestingly, the efficiency of remyelination also decreases with ageing and elimination of myelin debris is impaired in aged animals. We hypothesise that ageing impairs the ability of macrophages to phagocytose myelin debris. To test this hypothesis, we investigated the ability of macrophages from young and aged mice to phagocytose fluorescently labelled myelin debris. In addition we examined if immune rejuvenation improved the phagocytic capacity of aged mice for myelin debris. To this end, aged mice were irradiated and reconstituted with bone marrow cells from young or aged mice. Splenocytes from young, aged and chimeric mice were harvested, adhered to tissue culture plates and washed to enrich the percentage of splenic macrophages present. Fluorescently labelled myelin debris was added to these cultures and phagocytosis was analysed by flow cytometry. Myelin phagocytosis was significantly reduced in macrophages from aged mice compared to macrophages from young mice. Interestingly, levels of phagocytosis were partially restored in cells from aged mice reconstituted with young bone marrow. In conclusion, ageing decreases the ability of macrophages to phagocytose myelin debris. Our results may suggest that the ability of aged mice to clear myelin debris could be improved by rejuvenation of the immune system.

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Shift in the phenotype of infiltrating macrophages towards M2 subset is related to Bcl-2 expression in the myocardium during *Trypanosoma cruzi* experimental infection

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Infection with the cardiotropic parasite *Trypanosoma cruzi* causes Chagas' disease, the leading infectious cardiomyopathy in the world. Macrophage (MΦ) influx to the infected myocardium could participate either in defense/pathogenesis or repair mechanisms. The M1 pro-inflammatory phenotype (F4/80+ CD68+) participates in the elimination of the parasite whereas the M2 anti-inflammatory phenotype (F4/80+ CD206+) attenuates the inflammatory response and promotes tissue repair. We studied the kinetic of both infiltrating MΦ subsets by flow cytometry and the expression of the anti-apoptotic molecule Bcl-2 by western blot and inmunofluorescence in the myocardium of BALB/c mice infected with *T. cruzi* Tulahuen strain. We found a clear predominance of MΦ with M1 profile at 4 days post-infection (dpi) (84 ± 16% versus M2 16 ± 15%, $P < 0.005$). In contrast, at 7, 14, 21 and 23 dpi the M1 population diminished significantly (15 ± 7%, 6 ± 3%, 3 ± 3% and 0.8 ± 0.4% respectively) being strongly increased the M2 phenotype (85 ± 7%, 94 ± 3%, 97 ± 3% and 99.2 ± 0.4%, $P < 0.001$ versus M1 for each time). Moreover, an important F4/80+ CD68+ and CD206+ population was observed (77 ± 5%, 71 ± 6%, 71.3 ± 0.8%, 66 ± 10% and 69 ± 3% respectively). In addition, Bcl-2 exhibited a basal expression in control non-infected myocardial tissue, whereas it significantly increased at 21 and 24 dpi (3.7 ± 0.7 and 3.8 ± 1.3-folds; $P < 0.05$ versus control). This protein was mainly restricted to cardiomyocytes and, to a lesser extent, inflammatory cells.

Conclusion: A shift in the phenotype of infiltrating Ma from a predominant M1 towards M2 subset occurs during *T. cruzi* acute infection. In parallel with M2 appearance, Bcl-2 increased its expression in cardiomyocytes.

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PI3K/Akt pathway contributes to development of apoptosis resistance during differentiation of human macrophages by maintaining antiapoptotic Bcl-xL protein expression

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Macrophages represent long lived immune cells that are remarkably resistant to apoptosis, which allows them to perform in highly stressful environments. However, the signaling pathways that mediate the development of macrophage antiapoptotic phenotype during differentiation remain mostly unknown.

We have used THP1 cells and primary monocytes treated with PMA and MCSF respectively to generate human macrophages. By using chemical inhibitors for the PI3K and MAPK pathways, our results indicate that pretreatment of cells before differentiation with the PI3K/Akt inhibitor LY294002 and not other MAPK inhibitors induced caspase dependent apoptosis. LY294002 pretreatment before, not after differentiation, also resulted in decreased expression of antiapoptotic Bcl-xL protein. By using Akt specific siRNA we also show that inactivation of this signaling pathway leads to loss of Bcl-xL expression and apoptosis. Moreover, this effect was specific if Akt was knocked down before inducing differentiation with either PMA or MCSF, and not after cells had progressed through the differentiation process. Further investigation has shown that Bcl-xL expression is also dependent on NFkB in differentiating macrophages. However, NFkB activation was prevented by LY294002 pretreatment, indicating that NFkB is responsible for Bcl-xL expression via PI3K/Akt signaling.

These results indicate that survival of macrophages is distinctively regulated during and after differentiation. We have identified a signaling pathway consisting of PI3K/Akt activation of NFkB that is important in survival of differentiating macrophages by specifically sustaining antiapoptotic Bcl-xL expression. These results could provide therapeutic strategies aimed at eliminating cells when their survival is no longer beneficial for the host.

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Negative regulators of alveolar macrophages and their role in secondary bacterial complications following primary viral infection

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Background: Recent studies have shown that prolonged alteration in innate immunity following primary viral infection is an important factor in disease outcome. This alteration may be orchestrated by an up-regulation of negative regulators in innate immune effectors characterised by desensitisation of TLR.

Methods: Using a murine model, we assessed a panel of various negative regulators (e.g. TAM receptor family (Axl, Mer and Tyro3), Ron-β, ST2L, GRK2, Glucocorticoid receptor (GR), LXR-α/β and PPAR-γ) and their involvement in the over-regulation of alveolar macrophages following influenza infection. Axl is involved in broad desensitisation of TLRs upon activation. We focused on the expression profile of Axl and further characterised its expression on innate immune cells over 6 weeks after primary influenza infection.

Results: The expression profile of Axl on both alveolar macrophages and on neutrophils following influenza infection became significantly up-regulated, remaining higher than at its homeostatic level even 6 weeks after initial infection. Preliminary data also showed that the pre-treatment of alveolar macrophages with the Axl ligand GAS6 reduces their phagocytic activity.

Discussion: Up-regulation of Axl following a viral infection may explain the exacerbation of secondary bacterial complications

1 By its roles in desensitising TLRs on alveolar macrophages and,
2 By reducing the phagocytic activity of alveolar macrophages.

Antagonism of Axl may have relevant clinical applications.

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TSLP enhances iNKT cells proliferation and cytokine secretion

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Background: Invariant Natural Killer (iNKT) cells of the innate immune system recognise non-classical glycolipid antigens such as KRN7000 and have been suggested to form a bridge between innate and adaptive immunity by secreting cytokines which influence development of the adaptive response. These cells have been suggested to express the receptor for Thymic Stromal Lymphpoietin (TSLP), an Il-7 like cytokine, and an epithelial derived cytokine that has previously been shown to act on different types of immune cells and enhanceTh2 response.

Aim: To determine whether human iNKT cells express the TSLP receptor and characterise the effect of TSLP on iNKT proliferation and cytokine production.

Methods and results: PBMC were incubated withKRN7000, TSLP or KRN7000 + TSLP for 14 days. Cells then were double stained with Va24 and TSLPr antibodies at day 7 and day 14.There was an increase in iNKT cell number in the presence of TSLP at both time points from 0.43% to 33.22%. FACs analysis showed that TSLPr was expressed on iNKT. We found that all iNKT cells express TSLPr. We looked then at the effect of TSLP on cytokine expression and found that proliferating iNKT cells in the presence of TSLP and re-stimulating at day 14 for 48 h promotes secretion of IL-4 but levels of IFN-g production were reduced.

Conclusions: These findings suggest that TSLP can act directly on iNKT cells via TSLPr and support the suggestion that *in vivo* iNKT cells may act to skew the immune response towards TH2 in the presence of TSLP.

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Cot/tpl2 activity is required for TLR-induced activation of the Akt p70 S6k pathway in macrophages: Implications for NO synthase 2 expression

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LPS stimulation activates IKK and different MAP kinase pathways, as well as the PI3K-Akt-mTOR-p70 S6k pathway, a negative regulator of these MyD88-dependent intracellular signals. Here we show that Cot/tpl2, a MAP3K responsible for the activation of the MKK1-Erk1/2, controls P-Ser473 Akt and P-Thr389 p70 S6k phosphorylation in LPS-stimulated macrophages. Analysis of the intracellular signalling in Cot/tpl2 KO macrophages versus. Wt macrophages, reveals lower IkBa recovery and higher phosphorylation of JNK and p38a after 1 h of LPS stimulation. Besides, Cot/tpl2 deficiency further increases LPS-induced NO synthase 2 (NOS2) expression in macrophages. Inhibition of the PI3K pathway abolishes the differences in IkBa and NOS2 expression between Cot/tpl2 KO and Wt macrophages following LPS administration. Furthermore, in zymosan- and poly I:C-stimulated macrophages, Cot/tpl2 also mediates P-Ser473 Akt phosphorylation, increases IkBa levels and decreases NOS 2 expression. In conclusion, these data reveal a novel role for the Cot/tpl2 pathway in mediating TLR activation of the Akt-mTOR-p70 S6k pathway, allowing Cot/tpl2 to fine-control the activation state of other signalling pathways.

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Genetic regulation of early inflammatory responses shapes a pro-carcinogenic microenvironment in a *Helicobacter hepaticus*-driven innate colitis model

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A link between chronic inflammation and cancer development was shown for several disorders, including inflammatory bowel disease (IBD), which sets patients at increased risk to develop colitis-associated colorectal cancer (CAC).

Although it is known from GWAS that genetic predisposition plays a role in the etiology of colitis, genes involved in the development of CAC are not well defined. In our study we identified and fine-mapped a colitis susceptibility locus on mouse chromosome 3, which shapes inflammatory responses in the colon and thereby predisposes mice to later cancer development.

We identified a genetic interval that upon infection with *H. hepaticus* leads to colitis and splenomegaly in the susceptible mouse strain 129S6SvEv.RAG−/− but not in mice harboring a congenic chromosome 3 interval of C57Bl/6 mice (C3B.RAG−/−). Using a congenic approach we were able to narrow down the susceptibility region to a locus of 1.7 Mb. Recombinants containing this C3B region were not only protected from colitis but also showed a diminished frequency of *H. hepaticus* + AOM-induced invasive carcinoma. Reciprocal bone marrow chimeras revealed that this locus is acting in bone-marrow-derived cells. Dissection of early inflammatory responses showed that susceptible mice have an increased influx of granulocytes and higher levels of pro-inflammatory cytokines from as early as day 3 after *H. hepaticus* infection.

Taken together, the 1.7 Mb susceptibility locus directs a pro-carcinogenic environment through its actions in innate immune cells, where the accumulation of inflammatory cytokines and granulocytes could lead to increased DNA damage and eventually to cancer.

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Studies of the role of reactive oxygen species in degranulation of human neutrophils

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The NADPH oxidase of neutrophils (NOX2) is a complex enzyme consisting of membrane and cytosolic subunits. Upon activation the cytosolic subunits translocate to the membrane where the functioning oxidase complex is formed. The active enzyme transfers of one electron from cytosolic NADPH to molecular oxygen, generating thereby a variety of radical oxygen species (ROS), consisting an oxygen-dependent microbicidal system. However, the activity of NADPH oxidase is not restricted to destruction of invading microorganisms, but it can rapidly change the membrane potential causing a depolarization of membranes. We suposed that a membrane potential can participate in regulation of such a proinflammatory function of neutrophils as degranulation. In order to investigate that assumption, we tested the inhibitors of NADPH oxidase, diphenylene iodonium (DPI) and apocynin, in the *in vitro* model of CB/fMLP-activated neutrophils of healthy donors. Neutrophils were isolated by Ficoll gradient from fresh blood by venipuncture. Exocytic insertion of CD63 and CD66b into the cell membrane corresponding to azurophil and specific granules was determined by flow cytometry. ROS production was measured using luminol chemiluminescence method. It was shown that activation of neutrophils with CB/fMLP resulted in a high release of CD63 and CD66b on cell membranes, while adding of DPI and apocynin downregulated an exocytosis of both types of granules in a dose-dependent manner. The obtained data are discussed in the light of electrogenic activity of NADPH-oxidase, capable to cause the depolarization of neutrophils' membrane and to affect thereby a different proinflammatory functions, including degranulation.

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Validating *in vitro* chemiluminescence as a quantifiable predictor of *in vivo* acute inflammatory reaction

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Neutrophil ROS was used to predict the acute inflammatory response of tissue-based & synthetic biomaterials *in vitro* using chemiluminescent reporting and validated using a subcutaneous (SC) rat model.

Synthetic meshes; polypropylene, polyester terephthalate and polyglycolic acid of varying polymer conformation. Tissue-based implants; human and porcine materials from dermis and small intestinnal submucosa (SIS) varying in decellularisation and cross linking chemistries. Materials were incubated using continuous luminsecent recording with whole blood & pholasin, which emits photons in the presence of ROS. *In vivo* implants were delivered SC into 6 week old, male wistar rats. Sacrifices performed at days 2, 5, 7, 14 and 28 and observed using H&E staining after resin infiltraion, $n = 6$/mtaerial/timepoint. Statistics; Waller-Duncan Ranking

ROS demosntrated influence of material fabrication on leukocyte activation. Tissue-based implants; SIS more pro-inflammatory anatomical region than dermis, SDS most ROS stimulating deceullularisation reagent, cross-linking showed no effect on cell response. Synthetics; conformation more determining in cell response than polymer composition. It was possible to show inter-donor variation in material/ROS which varied as a function of time demonstrated using repeated blood collections. *In vivo*; validated histologically by extensive populations of polymorphonuclear cells interrogating the SIS and SDS materials compared to the remainder of the materials *in vivo*.

In vitro reporting of ROS demonstrated quantifiable prediction of biomaterial innate inflammation. The technique showed material fabrication parameters modified cell response & the extent of cell activation by material surfaces varies between individuals. A rat model validated these findings which concluded the technique an acurate representation of *in vivo* concequence.

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Species specific structural differences in CD1d determine the reactivity of iNKT cells to the endogenous glycolipid antigen iGb3

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Background: Based on studies in mice, isogloboside 3 (iGb3) has been claimed to be the major CD1d-presented self-antigen for iNKT cells. However, whether iGb3 is an important antigen for human iNKT is contentious, as humans lack key enzymes for iGb3 synthesis. Here we conducted a cross-species analysis of CD1d-mediated iGb3 presentation to iNKT cells.
Methods: Activation of human and mouse iNKT cells in response to iGb3, in the context of either human (hCD1d) or mouse CD1d (mCD1d), was examined using assays of *in vitro* expansion and cytokine secretion. Direct quantification of iNKT:CD1d binding was performed by both FACs and surface plasmon resonance using soluble recombinant CD1d and iNKT TCR proteins. Furthermore, loading of iGb3 onto CD1d was assessed using IB4, an iGb3-specific lectin. Site-directed mutagenesis was employed to examine possible species differences in CD1d-iGb3 presentation to iNKT TCRs.
Results: Neither human nor mouse iNKT cells were stimulated by iGb3 in the presence of recombinant hCD1d or hCD1d-expressing cells. Furthermore, human iNKT-TCRs did not bind to iGb3-hCD1d. In contrast, mCD1d was able to present iGb3 to human and mouse iNKTs. Changing a single amino acid in hCD1d, Trp153, to the mouse orthologue, Gly155, reversed this species difference in iGb3 presentation.
Conclusions: These data clearly demonstrate that iGb3 cannot act as an iNKT antigen when presented by hCD1d, and identify the molecular basis for this species difference. This resolves a controversial issue in the iNKT field, and enhances our understanding of important human-mouse species differences.

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A protective role for toll-like receptor 3 but not toll-like receptor 7 in intimal hyperplasia

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Introduction: Previous studies have assigned detrimental roles to toll-like receptors (TLR) in cardiovascular disease. Recently, we have shown that TLR3 plays a protective role in early atherosclerosis development in apolipoprotein E deficient mice.
Aim: Using murine models we investigated the consequence of TLR3 and TLR7 signalling in intimal hyperplasia.
Methods and Results: We examined the role of TLR3 and TLR7 signalling *in vivo* using a perivascular collar-induced injury model of neointima formation. No difference in neointima development was observed between untreated C57BL/6 wild-type and TLR3−/− or TLR7−/− mice. Surprisingly however, systemic administration of the dsRNA analogue poly(I:C) led to significantly reduced neointima formation in C57BL/6 mice in a TLR3 dependent manner ($P < 0.001$). In contrast, systemic administration of the TLR7/8 ligand R848 did not affect neointima formation in C57BL/6 mice ($P > 0.05$). Interestingly, genetic deletion of either TLR3 or TLR7 led to enhanced development of elastic lamina damage following collar-induced injury. Interruptions in the elastic laminae were wider and occurred more frequently in injured arteries of TLR3−/− and TLR7−/− mice compared to C57BL/6 mice. The occurrence of this phenomenon in the absence of an exogenous viral stimulus implicates endogenous vasculoprotective TLR3 and TLR7 ligands.
Conclusions: Collectively, our data reveal a protective role for TLR3 but not TLR7 signalling in intimal hyperplasia. However, both TLR3 and TLR7 appear to play a role in maintaining the integrity of the vessel wall through as yet unidentified endogenous ligands.

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The role of interleukin-1 receptor-associated kinases in toll-like receptor signaling pathway

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The adaptor MyD88 and Interleukin-1 receptor-associated kinases (IRAKs) play key roles in Toll-Like Receptor (TLR), IL-1- and IL-18-receptor-activated NF-κB signaling pathways. Whereas the functions of MyD88, IRAK1 and IRAK4 are well described, the detailed functions of IRAK2 and IRAK3 remain somewhat unclear. Crystallographic studies recently suggested that MyD88 triggers the incorporation of IRAK4 and IRAK2 into a higher order oligomeric signaling assembly, the so-called Myddosome. How different IRAKs fit into the structural context of the Myddosome and how Myddosome assembly is regulated remains unknown. Here we have investigated the contribution of MyD88 and different IRAK interactions using full-length (FL) and death domain (DD) version of each protein as well as naturally occurring mutations as functional probes in quantitative LUMIER interaction assays. Selected interactions were also tested using purified recombinant proteins and measured using biochemical and biophysical techniques. Our results point to a hierarchical assembly and defined rules for the incorporation of IRAK1 and IRAK3. Additionally, the IRAK kinase domains modulate the strength of Myddosome interaction. Finally, naturally occurring MyD88 mutations interfere with decisive steps in Myddosome assembly and may explain the observed associated loss-of-function effect and rare occurrence in the human population. In conclusion our studies shed light on the assembly of the Myddosome, a post-receptor complex that may be an attractive therapeutic target, for example in the treatment of certain lymphoma subtypes, where MyD88 or IRAK gain-of-function mutations drive cellular proliferation.

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Free-living mice display immune system traits that cast light on the traditional mouse model

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Despite millions of years of evolution of mammals in symbiosis with their microbiota, a large body of what we know about the immune system derives from mice kept under extremely hygienic conditions. The high level of inbreeding may also bias interpretation of results due to the limited diversity or the expression of abnormal recessive genes. We here present observations that free-living mice have activated natural killer (NK) cells and differences in gut mucosal architecture compared to C57BL/6 (lab) mice.

NK cells of free-living mice showed several phenotypic signs of a higher activation level (elevated CD69, Granzyme B, KLRG1, NKp46 expression), were more numerous in peripheral lymph nodes, were predominantly of the $CD27^{+}$/CD11b- subtype, and had quickly inducible CD25 expression and interferon-gamma production upon cytokine stimuli. These findings were clearly different from lab mice, cohering with recent hypotheses that NK cells need to be primed (or gain memory-like features) by microbial stimuli in order to reach full maturity, whereupon they enter lymph nodes and help Th1-cell type of immune responses.

Furthermore, histology of intestines reveals significantly different architecture in the GALT system of free-living mice as compared to lab mice. Some preliminary results will be presented.

These observations demonstrate that immunological traits may be significantly altered in mouse models removed from their natural environmental conditions, with the ensuing risk of misleading or undiscovered results in studies like vaccine or drug discovery. Further studies of wild mice are needed to clarify the roles of genetic versus environmental impact on these discrepancies.

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Immune adaptation in the central nervous system in response to systemic infections

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Systemic infection leads to the generation of inflammatory mediators that result in transient metabolic and behavioural changes. However, in experimental models of chronic neurodegeneration systemic inflammation is no longer harmless and increases neuronal damage through activation of 'primed' microglia. In this study we investigated the biological mechanism of immune adaptation of microglia and brain vascular endothelial cells in response to systemic bacterial infection.

Naïve mice or mice with a neurodegenerative disease were systemically infected with live *Salmonella typhimurium*. Inflammatory cytokines were measured in serum, spleen and brain, and microglia and endothelial cell phenotypes studied by immunohistochemistry. Microglia priming was assessed by intracerebral injection of LPS.

Serum cytokine levels (IFNγ, IL-1β, IL-6) peak at day 7 while brain cytokine levels (IL-1β, IL-12) continued to increase over 3 weeks in *S. typhimurium* infected mice. We observed persistent MHCII expression on cerebral endothelial cells, and transient changes in CD11b and CD68 expression on microglial cells. Intracerebral injection of LPS results in an exaggerated inflammatory response when compared to non-infected mice. Systemic infection of mice with ongoing neurodegeneration results in earlier impairment of motor function and increased activation of microglia when compared to mice that suffered from the neurodegeneration only.

These studies reveal that the innate immune cells in the brain do not become tolerant to systemic, real infections. This may lead to prolonged and damaging cytokine production. This lack of tolerance in brain tissue may have a profound effect on the progression of pre-existing neurodegenerative disease.

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Susceptibility of breast and ovarian cancer stem cells to cancer drugs and natural killer cells

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One of the reasons of tumor aggressiveness, metastasis and recurrence is the existence of cancer stem cells within the tumor. Natural killer (NK) cells are lymphocytes of the innate immune system that play a key role in direct elimination of transformed or infected cells. Recently, it has been reported that NK cells can get rid of cancer cells with the stem cell-like properties which can initiate the tumor formation. Here, we report that the isolated cancer stem cells sorted by cancer stem cell markers from the breast and ovarian cancer cell lines are more resistant to chemical drugs but susceptible to NK cell cytotoxicity compared with the cancer cells without cancer stem cell marker. In order to identify the reasons for the high susceptibility to NK cells, we examined the NK cell killing mechanisms against cancer stem cells. These findings proved NK cells effectively kill the drug resistant cancer stem cells, thus, offering NK cell immunotherapy could be useful in eliminating cancer stem cells and preventing tumor recurrence and metastasis.

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Mapping of RNA and imidazoquinoline sensing sites in the TLR7 and TLR8 extracellular domains

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Toll-like receptors (TLR) fulfil a critical role as innate immune sentinels against microbial invasion and different TLR are able to detect vastly different microbial molecules, for example nucleic acids of viral origin which are sensed by TLR3, 7, 8 and 9. Apart from viral RNA and its synthetic mimic, oligoribonucleotides (ORN), TLR7 and 8 also respond to small molecules of the imidazoquinoline (IMQ) family. Although human TLR7 and 8 are of high therapeutic interest, their principles of ligand recognition are poorly known. Here we focussed on determining recognition principles for both TLR7 and TLR8 ORN and IMQ ligands by combining homology 3D modelling and site-directed mutagenesis of the ectodomains (ECD) of TLR7 and TLR8. Our results suggest that charged residues in distal parts of the TLR7 and 8 ECDs, including the N- and C-termini, are vital for the recognition of both types of ligands. Interestingly, a subset of residues seems to discriminate between ORN and IMQ agonists. Our data are reminiscent of the recognition principles found in TLR3 and TLR9 and hints to a common mechanism of nucleic acid sensing by TLRs. Additionally, this sheds new light on the unique recognition of small IMQ by TLR7 and TLR8. Currently we are investigating whether ligand recognition residues also participate in ligand binding or whether recognition and binding are events mediated by different sets of residues. We consider answering this question and the development of reliable binding assays as vital for the efficient exploitation of TLR7 and TLR8 as therapeutic targets.

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In vitro, induction of BAFF and APRIL expression by A549 and BEAS-2B airway epithelial cells

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Background: Antibody production in the lungs is an essential defense mechanism against respiratory pathogens. However, little is known about the mechanisms of local activation of B cells in the airway mucosa.
Hypothesis and aims: The production of BAFF and APRIL by epithelial cells contributes to local accumulation, activation, class switch recombination, and antibody production by B cells in the airways. Here we aimed to characterise BAFF and APRIL production by the cultered airway epithelial cells, A549 and BEAS-2B.
Methods and results: *In vitro*, we investigated if cultured epithelial cells can be stimulated to produce BAFF or APRIL by viral infection, dsRNA or cytokines stimulation. A549 cells were stimulated with factors including, INFα, IFN β, IL-1 β, LPS and dsRNA (10–100 μg/ml) at different time points. Induced expression of BAFF and APRIL in A549 cells varied according to the stimuli used. For example, BAFF and APRIL mRNA were observed at 4 and 12 hrs post INF β stimulation. BAFF but not APRIL expression could be induced in Beas-2B cells, with IFN β 100 μg/ml at various time points or when infected with RSV A2 strain. When BEAS-2b cells infected with RSV were pre-incubated with anti-RSV Ab (Palivizumab) we found that expression of BAFF was blocked.
Conclusion: Collectively our results indicate that airway epithelial cells can produce BAFF in an interferon dependant manner. Suggesting that the Airway epithelial could help support B cell growth development and Ab production in the Lung.

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Regulation of toll-like receptors in porcine alveolar macrophages infected with porcine reproductive and respiratory syndrome virus

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a positive-single-stranded RNA virus of the Arteriviridae family. PRRSV causes significant losses to the swine industry worldwide. Infection with PRRSV predisposes pigs to infection by bacterial and other viral pathogens. PRRSV has a tropism for cells of a phagocytic lineage, especially porcine alveolar macrophages (PAMs). Toll-like receptor (TLR) ligands, basically TLR3, TLR7, and TLR9, are involved in innate immune responses by triggering the production of antiviral cytokines such as type-I IFN. Our objective was to determine whether different PRRSV strains regulate the expression of these TLRs in PAMs. Cultures of PAMs obtained from 4 week-old healthy pigs were infected with two field PRRSV isolates (IL-10^+/TNF-α^+ inducer and IL-10^-/TNF-α^- non inducer strains) and an attenuated vaccine at m.o.i = 0.1 and 1.0. Cells were harvested at different time-points post-infection (PI), and analyzed for the expression of TLRs and viral antigens by flow cytometry (FC) and real-time PCR (TaqMan®) respectively. Using FC, TLR3 increased in infected PAMs after 24–36 h PI, being this increase more evident in the case of the IL-10^+/TNF-α^+ isolate. TLR9 and TLR7 were also induced by the IL-10^+/TNF-α^+ strain at 48 h PI. The IL-10^+/TNF-α^+ isolate replicated at lower titers than the IL-10^-/TNF-α^- ($10^{6.0}$ versus $10^{7.3}$ TCID50/ml) and apoptosis was observed in a lower proportion of cells after 48 h of incubation. Also, clear-cut differences were more evident at m.o.i = 1. In conclusion, different PRRSV isolates can affect the TLRs expression in a different way and, consequently the development of innate immunity could be affected.

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Expression of the regulatory receptor CD200R on macrophages is regulated by polarization signals in atherosclerosis

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The glycoprotein receptor CD200R1 belongs to a family of four isoforms and signals by binding to its counterpart ligand CD200. The expression of the receptor is highly restricted to cells of the myeloid lineage such as macrophages and inhibits inflammatory signaling by blocking pro-inflammatory signals. Our work aims to determine the signals that regulate CD200R expression both *in vivo* and *in vitro* during atherosclerosis. Using an *in vitro* cell culture model with bone marrow derived macrophages from WT mice, we showed that the mRNA expression of CD200R1 is negatively regulated by polarizing macrophages with M1 signals such as Interferon gamma (IFN-g), Lipopolysaccharide (LPS) together or alone (up to 75% down-regulation, $P < 0.001$) or with synthethic diacylated lipoprotein (FSL-1). This pattern is followed by the M2 gene Mannose Receptor (CD206). Conversely, polarizing macrophages with M2 signals induced an increased expression of CD200R1 ($P < 0.05$), suggesting that CD200R1 is an M2 marker ($P < 0.05$). Furthermore, the study of CD200R$^{-/-}$ macrophages has revealed a lack of inflammatory control, both in terms of pro-inflammatory genes such as inducible Nitric Oxide Synthase (iNOS) when stimulated with M1 signals, but also anti-inflammatory genes such as IL-10 when stimulated with endogenous TLR ligands, such as POLY (I:C). Immunolocalisation studies in ApoE$^{-/-}$ mice have shown that CD200R is differentially expressed in secondary lymphoid organs during disease. In conclusion, a pro-inflammatory environment may lead to increased macrophage activation and disease progression, by inhibition of CD200R signaling.

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A novel role for CD46 in wound healing

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The complement regulator CD46 has recently gained interest among immunologists because it regulates cytokine production in human Th1 cells. Such immunomodulatory function is likely the reason as to why several important human pathogens use CD46 as cell entry receptor. Here, we present data suggesting that invading pathogens may take also advantage of an additional, novel role of CD46: We found that CD46 regulates epithelial cell barrier integrity and that activation of CD46 in intestinal epithelial cells accelerates wound healing.

Intestinal epithelial cells are crucial players in the induction of tolerance towards commensals and of inflammation against breaching pathogens. Because a role for CD46 in epithelial polarization has been previously described, we aimed at understanding the functional significance of CD46 expression in the gut epithelium. We observed that CD46 interacts with SPAK and α-E catenin, both proteins vital in the maintenance of transepithelial resistance, in intestinal epithelial cells. Further CD46 activation regulates the expression E-cadherin which is required for normal cell/cell junction formation. Engagement of CD46 on an intestinal epithelial cell line induced rapid decrease in trans-epithelial resistance, a concomitant increase of paracellular permeability and allowed for a significant surge in bacterial transgression. Importantly, the regulation/opening of cell junctions by CD46 activation induced epithelial cell proliferation and accelerated wound healing. Thus, CD46 plays a novel role in epithelial cell barrier maintenance and we are currently assessing for a contribution of deregulated CD46 signals/functions in disease states such as IBD and/or malignant transformation.

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Natural killer cell response to hepatitis C peptides

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Killer immunoglobulin-like receptors (KIR) keep both NK activation and inhibition in check through interactions with HLA Class I. It has been shown that individuals homozygous for both the inhibitory receptor KIR2DL3 and its HLA-C1 ligand have a higher frequency of resolution of Hepatitis C virus (HCV) infection whereas those homozygous for KIR2DL2 do not resolve infection. Recent work has showed that different variants of an endogenous peptide VAPWNSLSL, naturally eluted from HLA-Cw*0102, can induce different NK cell responses. Here, we investigated the effect that peptides derived from HCV may have on the NK response mediated *via* a KIR2DL2/3-HLA-C1 interaction.

Epitope prediction software was used to predict HCV-derived peptides that may potentially bind HLA-Cw*0102. Overall, nine nonamer HCV peptides with key anchor residues for binding HLA-Cw*0102 were synthesized. A TAP-deficient 721.174 cell line that expresses HLA-Cw*0102 was exogenously loaded with HCV peptides to check for stabilisation. Two of the nine HCV peptides stabilised HLA-Cw*0102 with affinities similar to that of VAPWNSLSL, and the remainder stabilised with much lower affinity. All peptides were tested in CD107a degranulation assays and one of the peptides LLPRGPRL had a significant inhibitory effect on KIR2DL2/3^{+} NK cell de-granulation when loaded on 721.174 cells. The remaining peptides had no effect on levels of NK cell degranulation.

These results show that the majority of HCV-derived peptides are non-inhibitory, and thus escape of HCV via the KIR:HLA system is rare. HCV is thus not well adapted to KIR2DL3, which appears to provide a selective advantage for the host.

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Different European strains of porcine reproductive and respiratory syndrome modulate apoptosis and necrosis during replication in bone marrow dendritic cells

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Despite the considerable number of published studies dealing with apoptosis caused by porcine reproductive and respiratory syndrome virus (PRRSv) at present it is unclear whether or not apoptosis occur in PRRSv infection. In this study, four European PRRSv strains (classified as regards the ability to produce IL-10 and TNF-a) were examined to assess the capability of the virus for inducing apoptosis and/or necrosis in porcine bone marrow derived dendritic cells (BMDC). Thus Il-10 antibodies were used to assess if blocking of this cytokine affected the induction of apoptosis. BMDC inoculated with non- IL-10 inducer isolates [3267 (IL-10^-, TNF-a^-) and 3249 (IL10^-, TNF-a^+)], showed about 40% and 28% of necrotic cells after 48 h post-inoculation, respectively. The proportion of apoptotic cells in these IL-10- non-inducers strains, were about 58% and 27%. In contrast, when IL-10^+ inducers strains [3262 (IL10^+, TNF-a^+) and 2988 (IL10^+, TNF-a^-)] were inoculated to BMDC, the average of necrotic cells were about 12.4% and 4.9%, respectively, and non apoptotic cells were detected. Moreover, when IL-10 was blocked in strain 3262 (IL10^+, TNF-a^+), 7.8% of apoptotic cells were detected. Finally, we note that virus viability was essential to induce apoptosis/necrosis. In this sense, heat inactivation of the virus does not produce any relevant level of apoptosis/necrosis. The results of this study suggest that IL-10 may play a protective role against the development of apoptosis and suggest that viral replication is needed to induce those mechanisms.

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The role of macrophages in biomaterial-tissue regeneration

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Extracellular matrix-derived (ECM) biomaterials exploit the body's regeneration capacity and are used to facilitate replacement of damaged or diseased tissue. Subcutaneous implantation of ECM biomaterials into a murine model showed infiltration of F4/80^+ cells, indicating that macrophages were amongst the first host cells to interact with the materials. The response of human macrophages to ECM biomaterials and the mode of regeneration of these implants are poorly understood.

To investigate the role of human macrophages in biomaterial integration an organotypic culture system was developed in which a decellularised porcine bladder matrix was maintained in close apposition to surgically-excised human urinary tract tissue. Human peripheral blood monocyte-derived macrophages were also studied on the decellularised biomaterial.

Histological examination of the biomaterial-organoids revealed a time-dependent emergence of cells expressing macrophage-associated markers CD68, HLA-DR and MAC387 at the wound-edge and within the scaffold. A striking and significant ($P < 0.05$) up-regulation of the haemoglobin scavenger receptor, CD163 was observed at the biomaterial-tissue interface compared to the central region of the tissue at days 6 and 11. By contrast, human monocyte-derived macrophages seeded onto a glass substrate or the porcine bladder matrix displayed a gradual loss of CD163 over the same period.

The macrophage has a coordinating role in wound healing and infiltration of these cells into the biological scaffold may promote recellularisation of the matrix. Expression of CD163 is associated with a regulatory macrophage phenotype, however, the driving force behind the up-regulation of CD163 and its functional role in biomaterial integration are not yet understood.

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Dendritic cell common G-chain cytokine receptor is required for optimal IL-15 transpresentation to CD4+ T cells at the immunological synapse

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Dendritic cell (DC) mediated trans-presentation of IL-15 in complex with IL-15Ra is known to be important for activation of NK and CD8+ T cells. Although growing evidence suggests a role for IL-15 in modulating CD4+ T cell function, it is unclear whether IL-15 trans-presentation is involved. Here, we show that trans-presentation of IL-15 by DCs is required for full antigen-mediated CD4+ T-cell activation and proliferation. We find that expression of the common cytokine receptor gamma chain (GC) on DCs is essential for effective IL-15 trans-presentation. Using high resolution imaging in combination with a planar lipid bilayer system that mimics the T cell surface, we demonstrate that DC-expressed gc is recruited to MHCII clusters at the immune synapse (IS) and mediates localization of IL-15Ra to the DC immunological synapse (IS). Our findings suggest a novel mechanism for DC CD4+ T-cell activation and a key role for DC-expressed gc at the immune synapse.

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CD141$^+$ (BDCA-3$^+$) interferon lambda (IFN-λ)-producing dendritic cells are depleted in hepatitis C virus-infected liver

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Dendritic cells (DCs) are critical to successful anti-viral immunity, being potent producers of interferon and key players in activation of adaptive immunity. Plasmacytoid DCs (pDCs) respond to, and produce IFN-λ (IL-28A, IL-28B, IL-29), a novel interferon family thought to have an important role in Hepatitis C virus (HCV) infection. IFN-λ is also produced by CD141$^+$ myeloid DCs (mDCs), in response to poly(I:C). However, little is known about which DC subsets are present in human liver, the primary site of HCV replication. In this study, liver perfusates were collected during transplantation and the DC populations and IFN-λ receptor expression were characterised by flow cytometry. Within liver Lin1$^-$HLA-DR$^+$CD11c$^+$ mDCs, CD141$^+$ DCs comprise a significant population (33%) whereas they account for only 6% of myeloid DCs in blood. Plasmacytoid DCs were significantly expanded in liver perfusate from HCV$^+$ donors ($n = 4$; mean 36.3%, range 32.5–40.1) compared to healthy perfusates ($n = 10$; mean 18.2%, range 9.2–27.2). CD11c$^+$ myeloid DC frequencies were similar in healthy and HCV-infected perfusates. However, CD141$^+$ DCs were significantly decreased in HCV-infected perfusate (15% versus 33%) whereas CD1c$^+$ (BDCA-1) DCs (43% versus 12%) were expanded. Increased IFN-λ receptor expression was observed on healthy liver perfusate cells compared to peripheral blood cells suggesting enhanced IFN-λ responsiveness in the liver. These results indicate that the liver is an important site of accumulation of CD141$^+$ DCs which are significantly depleted in HCV infection. The impact of the *IL28B* genotype on hepatic CD141$^+$ DC activity warrants further investigation.

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Low mannose-binding lectin in cord blood: is it a predictor of neonatal infection?

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Mannose-binding lectin (MBL) is an important serum protein of the innate immune response. MBL deficiency results in defective opsonization and phagocytosis especially in immunocompromised patients like neonates. The adaptive immune system of neonates, particularly of preterm infants, is severely impaired because of immature B and T cell function.

The aim of our study was to measure MBL concentration in cord blood of preterm and term neonates, to identify clinical characteristics associated with low neonatal MBL concentrations and to investigate whether low MBL levels are correlated to neonatal sepsis early and late onset or pneumonia during the first month of life.

Methodology: A cross sectional study involving 86 neonates delivered in Jeddah Clinic Hospital from March 2011 to May 2011. Ante- and intra-partum clinical data were recorded. MBL was measured in cord blood using sandwich enzyme immunoassay. When infection was suspected, complete blood picture, C-reactive protein and blood cultures were done.

Results: The median MBL plasma level was 1430 ± 560 ng/ml. Thirteen neonates (15.1%) had low MBL plasma levels ≤700 ng/ml. There was a significant direct correlation between MBL levels and both GA and birth weight ($P = 0.01$ and 0.03 respectively). There was a statistically significant difference between MBL deficient group and MBL non deficient group comprising low apgar score, early intubation and mechanical ventilation ($P < 0.05$). MBL deficient group was more susceptible for late onset infections ($P < 0.05$).

Conclusions: MBL is more deficient in premature neonates. It can be used as a predictor of pneumonia and sepsis in neonatal period.

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Human biotope probiotic bacterial lectins as signal system supporting biotope healthy balance

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Conceptions based on own results: 1 Probiotic bacterial lectins (PBL) of human probiotic bacteria are occurred and function as signal systems controlling pathogen survival in subagglutinating doses. PBL imitate probiotics (destruction of pathogen biofilms; antibiotic-like and distant pathogen growth inhibition, microcidic and lysis actions, cytokine inducing, immune correction, indirect protection of human antibodies against proteolysis by pathogen) and reveal anti-pathogen synergism in time (quick and delayed), space (symmetric or not, peripheral/border or central/comfortable for eukaryotic pathogen population) and stress: between bifidobacterial and lactobacillus L (BL, LL) together with antibiotics.

2 *Candida* populations act as communicative body interacting to both human systems and biotope microbiocenosis. *Candida* cells reveal high sensitivity to PBL (species and clinical strain discriminations are possible) resulting in final *Candida* body destruction. PBL and antibiotics give further phenotyping *Candida* clinical strain. Among *C. non-albicans*, interaction of *C. tropicalis* strains to BL orLL is more complex (potential involving hydrolases).

3 In biotope microbiocenosis containing PB and pathogens (like Staphylococcus and *Candida*), survival partially depends on supporting coupled antagonistic systems 'lactobacilli (completed by bifidobacteria) against staphylococci' and 'bifidobacteria (completed by lactobacilli) against fungi'. Being autostimulators, PBL will support probiotic microbiota in biotopes. In cases of *Candida* strains isolated from patient intestinal and urogenital biotopes, strain behavior in the presence of LB and/or LL is diagnostic.

Conclusion: Prospects of PBL as synbiotic/symbiotic ingredients are of importance for system anti-pathogen drug constructing.

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Aryl hydrocarbon receptor regulates skin immune responses

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The Aryl hydrocarbon receptor (AhR) recognizes polycyclic aromatic hydrocarbons, a family of structurally related environmental contaminants, and its ligation was shown to amplify the developmental program of Th17 cells and to induce IL-22 production. By being in constant contact with the environment, the skin is exposed to multiple pollutants that are potential AhR agonists. In addition, several endogenous ligands have been described recently, indicating a potential role for AhR in both skin homeostasis and immune responses.

We aim at describing skin immune responses in wild-type and AhR-deficient mice in order to assess the impact and integration of AhR activation in different skin cells. Taking into consideration the broad expression of AhR in the skin, which includes lymphocytes, monocytes, dendritic cells and epithelial cells, we will make use of cell-specific AhR-deficient mouse strains to study the effect of dietary, endogenous and environmental AhR ligands on different cell types and its consequences for the development of skin inflammation in response to injury or infection. Preliminary data indicates a dysregulation of the normal process of skin inflammation between AhR-deficient and wild-type mice, suggesting an important role of AhR in innate immune processes in the skin.

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Characterising dendritic cells in fish

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Dendritic cells present antigens to naïve T cells and are fundamental for the generation of adaptive immunity in mammals. Costimulatory signals received from dendritic cells and relevant cytokines are essential for the proliferation and generation of memory cells. Strong and long-lasting memory T cells responses hold the keys to successful vaccine development. Agents that activate dendritic cells and that can be used as molecular adjuvants are essential components for vaccine effectiveness, which in turn is important for disease control in aquaculture.

Detection of dendritic cells requires several surface markers since there is no marker exclusively expressed by dendritic cells. Two putative surface markers of dendritic cells, DC-SIGN (DC-specific ICAM-3-grabbing non-integrin)/CD209 and DC-LAMP (lysosomal-associated membrane protein)/CD208, have been identified and cloned in rainbow trout (Oncorhynchus mykiss), the DC-LAMP for the first time in fish.

To study whether DC maturation occurs in a similar manner in fish as in mammals, the expression patterns of DC-SIGN/CD209 and DC-LAMP/CD208 have been determined using mRNA quantification by quantitative RT-qPCR. A polyclonal antibody has been raised against the peptides derived from one putative DC-SIGN marker and the antibody will be used to isolate and further characterise the trout dendritic cells with techniques such as Western blotting, immunocytochemical staining and FACS analysis.

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Surfactant protein d interactions with rhinovirus

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Human rhinovirus (HRV) causes exacerbations in patients with chronic airway disease, hospitalisation of infants and pneumonia in the immunosuppressed. There is currently no treatment for HRV infections. Native human surfactant protein D (nhSP-D) is found in the lining fluid of the lung, it forms an important part of innate pulmonary immune defences. nhSP-D has anti-viral properties against influenza A virus and respiratory syncytial virus *in vivo*. A recombinant trimeric fragment of nhSP-D (rfhSP-D) has also been shown to have anti-RSV properties *in vivo*. Unlike nhSP-D, rfhSP-D can be expressed in high yield systems and therefore has pharmacological advantages over nhSP-D.

In the present study the hypothesis that rfhSP-D can be used as an anti-HRV therapeutic has been examined. Using an infection inhibition assay with monolayers of HeLa cells rfhSP-D was found to reduce the level of HRV-16 infection by 15% *in vitro*. Binding of rfhSP-D to HRV-16 was also observed using surface plasmon resonance, this was concentration and calcium dependent as binding was inhibited in the presence of EDTA. This data indicates that binding to HRV is via the carbohydrate recognition domain.

These results suggest that rfhSP-D may be a potential therapeutic against HRV infections. An anti-HRV therapeutic could have immense impact on treatment of patients with airway problems in which HRV triggered exacerbations can cause irreversible lung damage.

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Characterisation of bovine leukocyte Ig-like receptors

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Members of the Leukocyte Immunoglobulin-like Ig receptors (LILR), are innate immune receptors that have been shown to play a pivotal role in regulating both innate and adaptive immune functions, and are thus capable of influencing the host response to infection. LILR show more interspecies conservation than the closely related Killer Ig-like receptors, and homologues have been identified in rodents, primates, seals and chickens. The murine equivalents, paired Ig like receptors (PIR), contain two additional immunoglobulin domains, but show strong sequence and functional similarities to human LILR. The bovine genome was recently sequenced, with preliminary annotations indicating that LILR were likely to be present in this species. We therefore sought to identify and characterize novel LILR within the Bos *taurus* genome, compare these phylogenetically with LILR from other species and determine whether they were expressed *in vivo*. Twenty-six potential bovine LILR were initially identified using BLAST and BLAT software. Phylogenetic analysis using MEGA5 software, indicated that 16 of these represent novel bovine LILR. Protein structures defined using protein BLAST predict that the bovine LILR family comprises seven putative inhibitory, four activating and five soluble receptors. Preliminary expression analysis using Genome Analyzer IIx (Illumina) demonstrated that all 16 of these receptors are expressed *in vivo*. Our analyses have identified 16 LILR sequences in the bovine genome. The bovine receptor family appears to contain receptors which resemble the six domain rodent PIR as well as the four domain LILR found in other species indicating co-evolution of both receptor types within the same species.

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MVA85A: characterising the innate immune responses after vaccination and the effect of TLR1 levels on the acquired antigen-specific immune response

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MVA85A, a recombinant Modified Vaccinia virus Ankara expressing the *Mycobacterium tuberculosis* antigen 85A, can improve BCG induced protection in preclinical animal models and is highly immunogenic when administered to BCG vaccinated volunteers. The variation in the immune response to MVA85A in healthy volunteers however is extensive and we sought to investigate this variation in long-term immunogenicity by describing the early immune events following vaccination.

We analysed the changes in gene expression by DNA microarray on day of vaccination and 2 and 7 days later in 24 healthy volunteers vaccinated with 1×10^8 pfu MVA85A. MVA85A induces a strong pro-inflammatory response which is visible in unstimulated PBMC 2 days following vaccination. The interferon pathway, STAT signalling and NF-κB signalling are particularly upregulated. Several regulatory pathways including apoptosis and regulatory T cell signalling are also upregulated. This signature is evident 2 days after vaccination but has waned by day 7.

We have also found that toll-like receptor 1 (TLR1) levels on day of vaccination, both mRNA and protein, correlated with the long-term immune response to antigen 85A. This result has been validated in a second independent trial in which 12 volunteers were vaccinated with 1×10^8 pfu MVA85A. We are now further investigating the mechanisms of this relationship and its applicability to different vaccine regimes.

Cytokines and Chemokines

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CCR5-delta 32 allele is associated with the risk of developing multiple sclerosis in the Iranian population

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Introduction: The thirty-two base pair deletion on C-C Chemokine receptor 5 gene (CCR5-delta32) is known as a protective allele against disorders of immune system. We have studied this variation in Iranian multiple sclerosis (MS) patients and healthy controls.
Methods: DNA samples were prepared from whole blood of 224 patients with MS and 271 healthy controls. We amplified the fragment including CCR5-delta32 polymorphism and visualized the products in a documentation system after agarose gel electrophoresis. Data were analyzed using one way ANOVA and exact fisher's tests by SPSS-v13 and STATA-v8 programs.
Results: The delta32 allele was more frequent in ms patients compared with controls (OR = 2.5, $P < 0.0001$). Also we found a significant difference in frequency of delta32/delta32 genotype among patients and controls (OR = 8.2, $P < 0.0001$). Thjrity-three percent of patients with early disease onset were homozygote for delta32 allele, while this was only 17% in healthy subjects (OR = 2.4, $P = 0.007$). Furthermore, delta32 allele was more frequent among patients with early disease onset (OR = 2.2, $P = 0.0005$).
Conclusion: According to our study, delta32 allele on CCR5 gene might be a predisposing factor for developing MS in Iranian population and may be associated with lower age at onset. However, we didn't find any association between this polymorphism and the clinical course of the disease.

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Increased CCL20 and CCR6+ regulatory T-cell responses in the *Helicobacter pylori* infected human gastric mucosa

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Chronic *H. pylori* (*Hp*) infection recruits regulatory T-cells (Tregs) to the gastric mucosa. Peptic ulcer disease is more common in patients infected with *cag* pathogenicity island-positive (*cag*PAI$^+$) strains, and those with insufficient Tregs. A CCL20 chemokine response to *Hp* in the human gastric mucosa has been reported. Therefore, we investigated the mechanisms and hypothesised that gastric mucosal Tregs may express the CCL20 receptor, CCR6.

Gastric biopsies and peripheral blood were donated by 24 *Hp*+ and 34 *Hp*- patients attending the Queen's Medical Centre, Nottingham. CD4 cells were isolated, stained for Treg markers (CD25^{hi}, FOXP3$^+$, CD127^{lo}, Helios$^+$) and CCR6, before flow cytometry analysis. Biopsy cells and gastric epithelial cell lines (AGS, MKN28 and MKN45) were co-cultured with *Hp* strains for 24 h; CCL20 concentrations were assayed using ELISA.

Gastric biopsies from *Hp*+ patients secreted higher concentrations of CCL20 ($P = 0.015$). Co-culturing epithelial cell lines with *cag*PAI$^+$ *Hp*, but not *cag*PAI mutants, induced a dose-dependent increase in CCL20 production. In biopsies from *Hp*+ patients, 80−100% of Tregs expressed CCR6; CCR6$^+$ Tregs were present at 3.5-fold higher levels ($P = 0.050$). In peripheral blood from *Hp*+ patients, twice as many Tregs expressed CCR6 ($P = 0.021$).

We conclude that *Hp* induces CCL20 production by gastric epithelial cells in a *cag*PAI-dependent manner. In *Hp*+ patients, higher CCL20 levels were associated with greater numbers of mucosal CCR6$^+$ Tregs, and increased proportions of peripheral blood CCR6$^+$ Tregs. Migration assays will now be conducted *in vitro* and *in vivo* to determine the importance of CCR6-CCL20 interactions in the response to *Hp*.

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Strategies for the inhibition of chemokine (CCL2) mediated monocyte migration

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CCL2 mediated migration of monocytes has been shown to play an integral role in the pathogenesis of lethal reperfusion injury (LRI) following cardiopulmonary bypass operation.
Study aims: *In vitro* analysis of the properties of synthetic CCL2 inhibitors and GAG binding peptides in inhibiting CCL2 mediated monocyte migration, as potential therapeutics for the treatment of LRI.
Methods and results: THP-1 cells were used as a model of human monocytes. Chemotaxis assays were used in initial screening of the inhibitory effects of synthetic CCL2 inhibitor compounds (C1-8) and GAG binding peptides (P1-5) on (10 nM) CCL2 mediated monocyte migration. In the next stage of experiments the most potent compounds and peptides were tested using activated trans-endothelial chemotaxis (*in vitro* model of inflamed capillary wall) in the presence of 30 nM of CCL2. P1-5, C1 and C5 were most potent. The inhibitory effects of C5 on monocyte adhesion under flow and shear stress conditions was analysed using the Cellix system, with statistically significant reduction ($P < 0.05$) in adhesion to VCAM-1 coated channels in the presence of 10 nM of CCL2 and 50 μM of C5. Western blotting showed no inhibitory effects on CCL2 mediated monocyte expression of p-ERK1/2, following stimulation with 10 nM of CCL2 in the presence of C1 or C5.
Conclusion: The *in vitro* analysis of synthetic CCL2 inhibitors and GAG binding peptides has shown these strategies to be effective in blocking CCL2 mediated migration of monocytes. Further studies of the mechanism of action of these compounds will aid their development as anti-inflammatory therapies.

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Analysis of the molecular mechanisms involved in the antagonic functions displayed by a CCL13-chemokine derived peptide (CDIP-2)

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Progression in allergic asthma has been related with over expression of chemokines that induce eosinophil trafficking and activation. CCR3/CCL11, CCL13 chemokine axis has been widely recognized as a major component of eosinophilic inflammation and represents an important therapeutic target. CCL13 is a human CC chemokine chemoattractant for eosinophils, basophils, monocytes, macrophages, immature dendritic cells, T cells it shows important immunomodulatory activities on epithelial, muscular and endothelial cells; however is not clear what are the key residues in CCL13 that drives CCR3, 2 and 1 receptor activation.

We have previously reported that a synthetic peptide derived from CCL13 sequence (CDIP-2), has anti-inflammatory activities in a murine model of allergic airway inflammation induced with OVA (AAI). Further analysis of the mechanisms involved in the antagonistic functions has been shown that CDIP-2 reduces chemokine-mediated functions via CCR1, CCR2 and CCR3 receptors.

in vivo analysis of a chronic AAI model showed reduced total numbers of bronchi alveolar cells, peribronquial/perivascular infiltrate and lesser positive cells with F4/80+/CCR1+, F4/80+/CCR2+ and GR1+/CCR39+ markers after treatment. Finally we showed that CDIP-2 reduces Th2 cytokines in peribronchial lymph nodes cells *in vitro* challenged with OVA. Thus we showed for the first time that CDIP-2 has amino acids sequence maybe crucial for CCL13 receptors binding/activation. Furthermore we suggested that this strategy cold be use to generate more efficacious chemokine receptor antagonists based on the knowledge of specific ligand-receptor interactions.

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Application of an evidence biochip array for the simultaneous determination of cytokines related to inflammation

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Biochip array technology enables determination of multiple analytes with a single sample. Inflammation is a complex host defence response to injury, tissue ischemia, autoimmune responses or infectious agents and is mediated by a variety of soluble factors including cytokines. This study reports the application of an Evidence biochip array to the simultaneous measurement of cytokines related to inflammation.

This biochip array allows the simultaneous specific measurement of twelve cytokines: interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), epidermal growth factor (EGF), interferon γ (IFN-γ), monocyte chemotactic protein-1 (MCP-1), tumour necrosis factor α (TNF-α), vascular endothelial growth factor (VEGF). Simultaneous chemiluminescent immunoassays are employed. The Evidence analyser was used, this system automatically processes, reports and archives the generated data.

The sensitivity values of the immunoassays were 0.9 pg/ml (IL-1α, calibration range 0−500 pg/ml), 1.3 pg/ml (IL-1β, calibration range 0−250 pg/ml), 4.9 pg/ml (IL-2, calibration range 0−3000 pg/ml), 3.5 pg/ml (IL-4, calibration range 0−900 pg/ml), 0.4 pg/ml (IL-6, calibration range 0−900 pg/ml), 2.3 pg/ml (IL-8, calibration range 0−3000 pg/ml), 1.1 pg/ml (IL-10, calibration range 0−1000 pg/ml), 2.5 pg/ml (EGF, calibration range 0−900 pg/ml), 2.1 pg/ml (IFN-γ, calibration range 0−1500 pg/ml), 3.7 pg/ml (TNF-α, calibration range 0−1500 pg/ml), 25.5 pg/ml (MCP-1, calibration range 0−1500 pg/ml), 10.8 pg/ml (VEGF, calibration range 0−3000 pg/ml). All the immunoassays showed typical intra-assay and inter-assay precision expressed as %CV ≤ 15. Correlation of the biochip based immunoassays with commercially available immunoassays were performed in serum samples, linear regression on the resulting data generated $r > 0.85$.

Biochip array technology represents a useful tool for the generation of patient profiles of cytokines related to inflammation in clinical research settings.

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Defining the chemokine repertoire of the female reproductive tract in mice

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Leukocytes in the female reproductive tract (FRT) likely contribute to protection from infection, remodelling during the estrus cycle, implantation, placentation, labour, and post-partum resolution. Mechanisms governing leukocyte recruitment to the FRT are therefore likely to influence health, fertility, pregnancy and birth. Chemokines are key factors in driving tissue-specific leukocyte homing, yet little is known about their expression in the FRT. To begin to address this, we have characterised the chemokine profile of distinct anatomical compartments of the mouse FRT at each estrus cycle stage. Ovary, uterus, cervix and vagina were obtained from non-pregnant mice during proestrus, estrus, metestrus and diestrus, and expression of 34 chemokines and 11 chemokine receptors analysed using Taqman Low Density Arrays. Lung, skin, small intestine and colon were used as control tissues. This approach identified chemokines highly expressed in ovaries, cervix and vagina, and revealed that, within the FRT, the uterus shows unique expression of CCL28 and XCL1. Unexpectedly, estrus cycle stage had minimal effects on chemokine expression, although CCL7 was reduced during estrus and metestrus, and CCR4 dropped during diestrus. Expression of leukocyte markers were also analysed by qRT-PCR, and suggested that the uterus is the principal home of most leukocyte subsets within the FRT, while the vagina may be a particularly poor site for NK cell homing. This study provides a foundation for more detailed analyses of the role of chemokines in leukocyte trafficking to the FRT, and will aid studies aimed at defining the function of FRT leukocytes in female reproductive health.

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Zinc depletion induces interleukin-1β processing and release

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Zinc is an essential biological trace element required for proper immune functioning. Zinc deficient individuals have been reported to suffer compromised immune responses and increased levels of inflammatory cytokines. Inflammation is integral to the pathology of many disease states, ranging from pathogen dependent infectious disease to non-infectious disease such as cancer, heart disease, diabetes and stroke. One of the main mediators of inflammation is the pro-inflammatory cytokine interleukin-1β (IL-1β). Production of IL-1β occurs via a two step process; firstly the transcription of an inactive pro-form is initiated, followed by protease activation leading to the cleavage of IL-1β to a mature form. *In vitro* zinc depletion of macrophages, using the zinc chelators TPEN and DTPA, leads to pro-IL-1β cleavage and furthermore to increased release of active IL-1β. This would suggest that zinc depletion induces activation of proteases that cleave IL-1β. Levels of cellular labile zinc are tightly regulated by transient binding of zinc to cellular proteins. Ten percent of mammalian proteins have been proposed to bind zinc; although the specific effects of zinc deficiency on the majority of these proteins are unknown. By identifying a role for zinc depletion in IL-1β processing we move closer to identifying potential therapeutic targets for zinc deficiency induced inflammatory disease.

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The association of peripheral arterial disease (PAD) with infective and inflammatory factors: assessing the presence of chemokine receptors (CCR2, CX3CR1 and RANK)

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The predominant pathologic feature in PAD is atherosclerosis which is an inflammatory condition. Diabetes is also considered as an inflammatory condition, and having both diabetes and PAD results in severe PAD complications that increase the risk of lower limb amputations. Our study focused on infective agents which may propagate the inflammatory condition. Studies have indicated a role of *Chlamydia Pneumoniae* and *Mycoplasma Pneumoniae* in atherosclerosis formation.

We would like to assess a number of relevant chemokine receptors involved in propogation of atherosclerosis and arterial calcification such as CCR2, CX3CR1 and RANK. These inflammatory markers will be assessed by

1 Examining their presence in arterial segments derived from amputated PAD samples by immunofluorescence staining and

2 Flowcytometry assessment of cells derived from PAD arterial segments for up regulation of pro-inflammatory chemokine receptors with and without LPS activation.

We found that PAD samples with diabetes tend to have severe lesions associated with arterial calcification and all PAD samples have a moderate to severe degree of atherosclerosis. Presence of RANK is in concordance with the calcified lesions in a number of PAD samples. While presence of CX3CR1 is in concordance with the atherosclerotic features. The early marker for atherosclerotic changes (CCR2) was not present and also minimally induced by activation with LPS. Both RANK and CX3CR1 surface expression were otherwise enhanced with LPS activation.

Our studies suggest a plausible role of bacterial infections in arterial changes due to inflammation. PAD complications arising from severe inflammation may contribute towards higher risk of amputations.

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IL-27 regulates IL-18 binding protein (BP) in skin resident cells

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IL-18 is an important mediator involved in chronic inflammatory conditions such as cutaneous lupus erythematosus, psoriasis and chronic eczema. An imbalance between IL-18 and its endogenous antagonist IL-18 binding protein (BP) may account for increased IL-18 activity.

IL-27 is a cytokine with dual function displaying pro- and anti-inflammatory properties. Here we provide evidence for a yet not described anti-inflammatory mode of action on skin resident cells. Human keratinocytes and surprisingly also fibroblasts (which do not produce any IL-18) show a robust, dose-dependent and highly inducible mRNA expression and secretion of IL-18BP upon IL-27 stimulation. Other IL-12 family members failed to induce IL-18BP. The production of IL-18BP peaked between 48 and 72 h after stimulation and was sustained for up to 96 h. Investigation of the signalling pathway showed that IL-27 activates STAT1 in human keratinocytes and that a proximal GAS site at the IL-18BP promoter is of importance for the functional activity of IL-27.

The data supports a significant anti-inflammatory effect of IL-27 on skin resident cells. An important novel property of IL-27 in skin pathobiology may be to counter-regulate IL-18 activities by acting on keratinocytes and importantly also on dermal fibroblasts.

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The immunological significance of the ST2L/IL-33 interaction in obesity-associated inflammation and diabetes

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Low-grade chronic inflammation is considered as a major determinant governing obesity and its progression to insulin resistance and type II diabetes. Recent animal studies suggest that Th1 cells and cytotoxic T cells are involved in the attraction of proinflammatory adipose tissue macrophages, which are a major cause of adipose tissue inflammation, and that Th2 cells are protective from such inflammation. The ST2L molecule is expressed on the surface of macrophages, monocytes and Th2 cells. The ligand for ST2L is IL-33. It has been shown that murine IL-33 acts as a chemoattractant for Th2 cells and induces Th2-associated cytokine production. In addition, IL-33-treated DCs stimulate naive $CD4^+$ T cells to produce robust IL-5 and IL-13. Therefore, ST2L/IL-33 interaction may play a critical role in the induction, maintenance and recruitment of protective Th2 cells in the adipose tissue. However, the role of the ST2L/IL-33 pathway in obesity-associated adipose tissue inflammation and diabetes has not been determined. We aimed in this study to compare the expression level of ST2L and IL-33 in lean and obese individuals with and without diabetes.

Plasma, serum, peripheral blood mononuclear cells, and adipose tissue biopsies were isolated from adult lean and obese subjects with and without diabetes, and assessed using immunological assays such as ELISA, Flow Cytometry and Immunohistochemistry. Data on the expression level of ST2L and IL-33 will be presented. Understanding of the significance of the ST2L/IL-33 interaction may help in the prevention of obesity-associated adipose tissue inflammation and hence diabetes.

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Chemokine-mediated preferential recruitment and subsequent selective retention account for the enrichment of CD4+Foxp3+ regulatory T cells in Methycolanthrene-induced tumors

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The frequency of CD4+Foxp3+ regulatory T cells (Treg) is significantly increased in peripheral blood and within tumours of cancer patients and mice. Enhanced proliferation and to a lesser extent conversion of conventional CD4+ T cells (Tconv) into Treg have been suggested to account for this. However, chemokine-induced migration and subsequent retention of Treg within tumors is not well documented.

Using a highly validated RT-PCR array we identified several chemokines strongly expressed by Methylcolanthrene-induced tumors, namely: CCL2, CCL5, CCL7, CCL8, CCL12, CX_3CR1, CXCL9, CXCL10, CXCL12 and CCL21. Analysis of corresponding chemokine receptor expression by flow cytometry revealed that CCR1, CCR2, CCR3, CCR4, CCR5, CX_3CR1, CXCR3 and CXCR4 were predominantly expressed on tumour-infiltrating CD4+ T cells compared to cells isolated from spleens and lymph nodes. Side by side comparisons of tumor-infiltrating CD4+ T cells showed that significantly higher proportions of Tconv expressed CCR1, CCR2, CX_3CR1 and CCR7 than Treg, while significantly higher proportions of Treg expressed CXCR3 and CCR4. However, in chemotaxis experiments Treg displayed greater migratory capability than Tconv which was augmented in the presence of specific chemokines. Further characterization indicated that over 60% of tumour-infiltrating Treg were $CD44^{hi}CD62L^-CCR7^-CD103^+$, a phenotype associated with retention within inflamed peripheral tissues due to down-regulation of major lymph node homing molecules.

Thus, tumor-expressed chemokines recruit both Tconv and Treg, but expression of CXCR3, CCR4, CD44 and CD103 by more Treg coupled with their superior migratory capacity confer a migration advantage that allows early and rapid migration of Treg to the tumor where they are retained.

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Retinoic acid enhances IL-22 production by innate lymphocytes and protects against colitis

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IL-22 plays a critical role in maintaining intestinal epithelial cell integrity and thereby protects against colitis. We have found that mouse $\gamma\delta$ T cells produce innate IL-22 and IL-17 in response to IL-23 in combination with IL-1β or IL-18, without TCR engagement. Retinoic acid (RA) also plays an important role in regulating intestinal homeostasis, by inducing lymphocyte mucosal homing receptors, enhancing $Foxp3^+$ Treg cells and suppressing development of Th17 cells. Recent studies have also shown that in the appropriate environment, for example during infection or in autoimmune diseases, RA can enhance Th cell responses. The effect of RA on $\gamma\delta$ T cells and innate lymphoid cells (ILCs) remains to be defined. We found that stimulation with IL-23 and IL-1β induced expression of RA receptor (RAR)-α and RAR-γ in $\gamma\delta$ T cells. RA inhibited IL-23 and IL-1β-induced IL-17 production by $\gamma\delta$ T cells *in vitro*, but surprisingly enhanced IL-22 production. In contrast, RA had no effect on IL-22 production by $CD4^+$ Th cells. ILCs in the lamina propria are another important source of IL-22, and we found that RA also enhanced IL-22 production by lamina propria cells. In a murine model of colitis, treatment of mice with RA attenuated clinical symptoms of disease and significantly enhanced IL-22 production in the colon, but did not affect production of IL-17 and IFN-γ. These findings suggest that $\gamma\delta$ T cells and other ILCs are important sources of innate IL-22, which can be regulated by RA to protect against intestinal inflammation.

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Dendritic cell (DC) $P2X_7R$ expression and interleukin (IL)-1β responses

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The $P2X_7R$ is a functionally distinct member of the P2X ionotropic family of receptors and has been implicated in the initiation of immune responses. The receptor is primarily involved in the release of IL-1β, however, previously attention has focused primarily on macrophages. The purpose of the current investigations was therefore to compare $P2X_7R$ activation in mouse bone marrow derived (BM) DC with responses in peritoneal macrophages (PMΦs). Using western blotting, both populations have were found to express $P2X_7R$, whereas unfractionated spleen cell preparations were largely negative. Treatment of immature BMDCs with lipopolysaccharide (LPS) (2 h) caused a dose dependent up-regulation of IL-6 but not of IL-1β. Rapid (2 h) IL-1β release required LPS priming and ATP activation both in BMDCs and PMΦs, however, markedly higher levels of IL-1β were released from BMDCs compared with PMΦs. In BMDCs, this rapid IL-1β release (but not IL-6) was potently inhibited with a $P2X_7R$-specific inhibitor (A740003) providing evidence that is predominantly a $P2X_7R$-driven process. Furthermore, IL-1β release was detected following long-term (24 h) culture of BMDCs (but not PMΦs) with LPS in the absence of exogenous ATP and this was inhibited potently with A740003. In conclusion, it is clear that there are some subtle but intriguing differences in the mechanism of $P2X_7R$ activation and IL-1β release between DCs and macrophages.

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A single dose of anti-mouse IFNg reduces key clinical and laboratory features of hemophagocytic lymphohistiocytosis (HLH) in a mouse model

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Hemophagocytic lymphohistiocytosis (HLH) is an immune response deficiency characterized by the hyperactivation of immune cells including T cells and macrophages. The disease leads to hyperproliferation, hypercytokinemia, aberrant tissue infiltration, pancytopenia and uncontrolled phagocytosis of erythrocytes, platelets and leukocytes. LCMV-infected perforin-deficient ($pfp^{-/-}$) mice develop the typical clinical and laboratory features of HLH, and support the essential role of $CD8^+$ T cells and IFNγ in this disease: these mice develop fever, splenomegaly, pancytopenia, hypertriglyceridemia, hypofibrinogenemia and hemophagocytosis. Importantly, these features as well as serum cytokine profiles in this murine model are similar to patients with HLH who exhibit drastically elevated TNFα, IL-6, IL-10, and IFNγ levels. Using LCMV-infected $pfp^{-/-}$ mice, we demonstrate that the administration of a single dose of the rat anti-mouse IFNγ, XMG1.2, at 30 mg/kg is capable of reversing key clinical and laboratory features of HLH. Several physiological parameters including weight, temperature, ferritin, triglycerides, cytokines levels and white blood cell counts are normalized following treatment. In parallel, we show that a XMG1.2 serum concentration of around 45–250 nM appears to saturably neutralize IFNγ. The data obtained in this mouse model of HLH are essential to design the development strategy of our therapeutic IFNγ antibody, NI-0501.

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The inflammatory mediators, osteopontin and IL-18, are increased in obesity: association with insulin resistance in obese individuals

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Obesity is associated with adipose tissue inflammation that is involved in the development of insulin resistance and type 2 diabetes. Osteopontin (OPN) and IL-18 are known to have potent inflammatory functions and both participate in a wide range of biological processes linked to immunological disorders. Since an interaction between OPN and IL-18 has been suggested in inflammatory diseases, we investigated whether:

1 Their levels were elevated in obese individuals;
2 Associated with blood glucose and BMI.

PBMCs and plasma samples were isolated from 77 individuals including lean as well as overweight and obese, with or without diabetes. Plasma concentrations of OPN and IL-18 were measured by ELISA. OPN and IL-18 mRNA expression was quantified in PBMCs by RT-PCR. *As* compared with lean controls, obese individuals showed significantly higher plasma concentrations of OPN (lean 2171 ± 203 pg/ml versus obese 2865 ± 101 pg/ml; $P < 0.002$) and IL-18 (lean 308 ± 45 versus obese 629 ± 96 pg/ml; $P < 0.01$). OPN showed a significant positive correlation with BMI ($P < 0.0001$) and blood glucose level ($P < 0.03$). Similarly, IL-18 positively correlated with BMI ($P < 0.05$) and blood glucose level ($P < 0.05$). Interestingly, there was a strong association between OPN and IL-18 in obese individuals. To our knowledge this is the first demonstration showing that plasma OPN and IL-18 are simultaneously increased in overweight/obese individuals which may trigger obesity-associated/insulin-resistance development. Further studies are being carried out to dissect the pathways that involve OPN and IL-18 in obesity.

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Interleukine-12/interleukine-4 in human echinococcosis: immunomodulation and new perspectives in anti-hydatic treatment

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Human hydatidosis is a widely endemic helminthic disease, caused by the larval stage of metacestode *Echinococcus granulosus*. It constitutes a serious public health problem in various parts of the world, particularly in Algeria. The lack of anti hydatic molecules which reduce the risk of relapse during hydatidosis and in the basis of our previous studies showing the protective role of Th1 cytokines, especially IFN-γ and IL-12 (Mezioug and Touil-Boukoffa., 2009; Mézioug and Touil-Boukoffa., 2005*), we investigate IL-12 effect on protoscoleces of Echinococcus granulosus (larval form of parasite) cultures in presence of peripheral blood mononuclear cell from hydatid patients. The evaluation of cytokines in supernatants of PBMC cultures revealed significant levels in IFN-γ in relation with low levels of IL-4. Our results show that in addition to stimulating effect on IFN-γ, production, IL-12 has negative regulatory effects on Th2 cytokines synthesis in co-cultures. We have noted with interest that co-cultures treatment with IL-12 caused an increased NO production and a decrease in the percentage of viable protoscoleces. However, the treatment of co culture with IL-4 reduced NO level and enhanced protoscolices viability and arginase activity. These results were confirmed by an inverse effect after neutralization of IL-12 and IL-4 effect by specific monoclonal antibody. Collectively, our results open perspectives on understanding the mechanisms of resistance and pathogenesis observed in human hydatidosis and allows considering new therapeutic strategies.

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Cytokine signalling is modulated by a labile disulfide Bond in CD132

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Some disulfide bonds in leukocyte membrane proteins are labile and can be modified in immune responses. We show that a disulfide bond in CD132, the common gamma chain of the receptors for several cytokines including IL-2 and IL-4, is susceptible to reduction by mild reducing conditions comparable with those found in immune activation. This disulfide bond is exposed at the surface of CD132 and in close contact to IL-2 and IL-4 in their respective receptors. Mutations in Cys residues that make this disulfide bond lead to loss of function. These mild reducing conditions inhibited the proliferation of an IL-2 dependent T cell clone but had no effect either on an IL-2 independent T cell clone or on the disulfide bonds of IL-2 itself. CD132 was identified in a screen for proteins with labile disulfide bonds in spleen following inflammation induced by lipopolysaccharide suggesting that the modification of CD132 is likely to occur *in vivo*. These results may have wider implications for the regulation of cytokine receptors in general as the activity of cytokine receptors may be modulated by a 'redox regulator' mechanism caused by changes in the redox environment during inflammation.

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A tissue-restricted antiviral function of interleukin-22 in murine cytomegalovirus infection

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Interleukin-22 (IL-22) is an IL-10 cytokine family member that signals through the IL-22RaIL-10Rb dimeric receptor. IL-22 is expressed by hematopoietic cells, whereas IL-22Ra expression is restricted to non-hematopoietic cells with elevated expression in peripheral tissues, particularly at barrier surfaces such as the skin, oral/gastrointestinal tract and lung. Initial studies have demonstrated that IL-22 affords tissue protective functions and contributes to immune control of gram-negative bacterial infections of mucosal surfaces. However, the role that IL-22 plays in acute and chronic viral infections is not understood. Utilizing the murine cytomegalovirus (MCMV) model, we have discovered that IL-22 affords antiviral protection *in vivo*. MCMV infection was associated with IL-22 protein secretion in mucosal (lung) and non-mucosal (spleen, liver) sites of infection. Following IL-22 neutralization, we observed a tissue-restricted elevation of acute virus load with increased virus burden in the lung and the liver but not spleen. Given the known importance of IL-22 in orchestration of mucosal immune responses, we further examined the lung. Elevated pulmonary virus load was associated with a reduced infiltration of neutrophils during acute infection. The role that neutrophils play in MCMV infection will be discussed. In summary, we demonstrate for the first time that IL-22 affords protection from a viral infection.

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Cytokine requirements in models of inflammatory bowel disease

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The increasing prevalence of inflammatory bowel diseases in the developed world reinforces the need to understand the mechanisms that lead to onset of inflammation in the gut. Genome-wide association studies showed a correlation between a SNP in the IL-23 locus and IBD. IL-23 is a pro-inflammatory cytokine that maintains IL-17-producing effector T cells which can cause pathology. However, mouse models where no T or B cells are present can still develop colitis in an IL-23-dependent manner, indicating that IL-23 can also act on innate cells of the immune system to drive disease, and we previously showed that a population of IL-23-responsive innate lymphoid cells (ILCs) could drive inflammation. Similar to lymphoid tissue inducer cells, these ILCs express receptors for several of the gamma chain family of cytokines, including IL-7, IL-2 and IL-15, many of which are typically considered important for cell survival.

To understand the other cytokine requirements of ILCs we have used models of innate pathology where gamma chain cytokines were modulated, and investigated the effects on colitis. Interestingly, different models of colitis showed different dependence on gamma chain cytokines. Removal of IL-7 or IL-15 signals affected the pro-inflammatory cytokine profiles in the gut, but in alternative ways. ILCs also responded differentially to gamma chain cytokines. Our results suggest a complex network of cytokines regulates colitis and that 'homeostatic' cytokines can play an additional role in enhancing the pro-inflammatory cytokine mileau associated with IBD.

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The role of infective and inflammatory factors (CCR2, CX3CR1 and RANK) in peripheral arterial disease (PAD) of the lower limb in diabetic and non-diabetic patients

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Atherosclerosis and calcifying changes of the arterial wall are the predominant pathologic features associated with inflammation in peripheral arterial disease (PAD). The cause of PAD is not known but a number of infective bacterial agents have been associated with arterial diseases. Our study focused on detecting the presence of *Chlamydia Pneumoniae* and *Mycoplasma Pneumoniae*, as well as on expression of pro-inflammatory receptors, including CCR2, CX3CR1 and RANK in pathological arteries. CCR2 is considered as an early marker for atherosclerotic changes, while CX3CR1 may promote smooth muscle cells proliferation. RANK, however, may be involved in arterial calcification.

Arterial samples ($n = 31$) were collected from 10 amputated limbs due to severe PAD and assessed for the presence of infective agents and inflammatory markers using immunofluorescence microscopy. Primary smooth muscle cell lines were established and used for detection of pro-inflammatory chemokine receptors with and without LPS activation using flowcytometry.

For all study subjects, the entire arterial sample showed atherosclerotic changes, as well as evidence of one or both bacterial infection. PAD samples derived from diabetic patients (6/10) displayed more severe lesions with calcification. CCR2 was not present in any of PAD samples and also minimally induced by activation with LPS. Both RANK and CX3CR1 surface expression were enhanced with LPS activation and these receptors were found to be expressed in tissue samples.

Our studies suggest a role of bacterial infections inducing inflammatory changes in PAD lesions. The presence of RANK and CX3CR1 are consistent with the calcification and atherosclerotic features present in the samples.

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Identification of a dominant T cell subpopulation in the uterine horn during postpartum remodelling

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Extensive uterine remodelling in humans is essential after birth to ensure future reproductive health. We have previously demonstrated that labour is associated with an extensive inflammatory influx, and sought to further characterise the cellular and chemokine profiles during the resolution phase. Time mated wild type mice were assessed during labour and then in the post-partum period on day 1, day 4, day 7 and on completion of several breeding cycles. qPCR characterisation of cell specific markers demonstrated that T cell specific markers initially increased on day 1 postpartum and attained peak levels on day 4 with maintenance thereafter. Macrophage markers, F480 and CX3CR1, demonstrated similar increases, while the neutrophil marker Gr1, declined from the peak attained during labour, with further decreases thereafter. Temporal analysis of chemokine receptor expression demonstrated a contemporaneous postpartum increase in CCR2 and its ligand CCL3. Flow cytometry was used to further characterise T cells in the uterine horn, cervix and peripheral tissues. There was a substantial increase in uterine and cervical $CD45^+$ cells and $CD45^+$ $CD3^+$cells on day 1 postpartum. Further characterisation demonstrated that these were principally of the $CD3^+CD4^-CD8^-$ phenotype, with associated increases in proportion of these cells in the circulation. Assessment of fluorescent CCL2 uptake suggested potential presence of CCR2 on these $CD3^+CD4^-CD8^-$ cells. Repeat analysis in CCR2 deficient mice on day 1 post-partum demonstrated equivalent uterine expression suggesting that alternative pathways were responsible for trafficking of this dominant T cell population which potentially has a unique regulatory role in the genital tract.

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CXCR3 dependant recruitment and CCR6 mediated positioning of Th17 and Tc17 in hepatic inflammation

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Introduction: Th17&Tc17 are distinct lineages of T lymphocytes implicating autoimmunity, bacterial infections in murine models and human diseases. However little is known about phenotype of liver infiltrating (LI) Th17&Tc17 cells, recruitment mechanism, positioning in the liver.

Methods: Human LI lymphocytes were isolated from explanted liver tissue. LI-Th17&Tc17 cells frequencies, chemokine receptors expression, and cytokine secretion assessed *ex-vivo* by flow cytometry, distribution in tissue determined using immunohistochemistry. Recruitment was studied *in-vitro* using flow-adhesion assay, *in-vivo* by IVM in ConA&CCL4-murine liver injury. Migration was investigated by transwell chemotaxis assay.

Results: Inflamed human liver contains a higher proportion of LI-IL-17 cells comprising 3% of T cells infiltrate with associated neutrophils. LI-Th17&Tc17 were restricted to $CD161^{high}$, CD45RO, express RORc, IL-23 receptor and secrete IL-17&22, IFN-g. Human Th17/Tc17 cells and recruitment via sinusoids is dependent on CXCR3, ICAM-1, VCAM, and VAP-1. Murine Th17 cells recruitment was investigated by IVM and CXCL-10 block inhibits recruitment. LI-IL-17 cells were detected around intrahepatic bile ducts. CCR6 ligand, CCL20 was restricted to bile ducts and cytokines stimulated human cholangiocytes secrete CCL20 which regulates *in-vitro* migration of cells towards cholangiocytes. Confocal microscopy detected LI-IL17 resides closely with regulatory T cells.

Conclusion: We report a novel subset of intrahepatic Th17&Tc17 subset cells. CXCR3 is critical in recruitment via hepatic sinusoid to the inflamed liver in both human and murine model whereas functional CCR6 localises to the inflamed intrahepatic bile ducts, which secrete CCL20. These IL-17 secreting cells also link the innate and adaptive immunity by recruiting neutrophils to site of inflammation.

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Immune cellular response to *Dermanyssus* infestation in poultry birds

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Dermanyssus gallinae is a haematophagous ectoparasite of bird and mammals including humans. The objective of the current study was to determine the initial immune response in poultry birds exposed to *Dermanyssus gallinae* by measuring mRNA expression.

Mite chambers were secured to birds in two treatment groups (12 birds/group), Control and Infested. Controls received no mites, whilst infested birds received 200 unfed female *D. gallinae* on Day 0. Mites were removed on Day 1 or 2 and birds were euthanased on Days 1, 2 and 5. The expression of Th1 (IFN-γ and IL-18), Th2 (IL-10 and IL-13) and pro-inflammatory cytokines (IL-6 and CXCLi2 (the avian equivalent of IL-8) in spleen samples from three out of four birds/group at each time point was determined by semi-quantitative PCR.

IFN-γ, IL-18, IL-6 and CXCLi2 expression was increased slightly on Day 1 (1.79, 1.45, 1.26, 1.28, respectively), whilst on Day 2 the expression level of these cytokines was reduced to below that of the Control group (0.53, 0.61, 0.85, 0.54, respectively). On Day 5, IFN-γ, IL-18, IL-6 expression was elevated (1.13, 1.10, 2.43, respectively) whilst CXCli2 was the same between groups (1.04).

Data suggest that *D. gallinae* feeding stimulates Th1 and pro-inflammatory cytokines initially followed by their subsequent down regulation.

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Identification of putative biomarkers in cancer therapy

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A recent clinical trial of dendritic cell (DC) vaccination in late stage melanoma has shown elevated markers of inflammation in the serum of patients not responding to therapy. Additional biomarkers have been identified using *in silico* methods, which are linked to inflammation and have immuno-modulatory abilities.

Briefly, pre-vaccination sera were analysed for a large range of inflammatory molecules using cytometric bead array technology. Increased levels of apoC3, TNF α, SCF, MiP1 α and IL-12p40 were seen in patients not responding to the therapy. An *in silico* platform was then employed to identify additional novel biomarkers. Using Ariadne Pathway Studio software we identified a number of potential markers with a high level of connectivity between identified inflammatory markers and their regulators. One of these putative markers, Apolipoprotein E (ApoE) was then assayed in the patient serum using ELISA and shown to be significantly increased in the non-responding patients compared to the responders.

ApoE has been shown to modulate immune cell function and to regulate tumour progression. ApoE mediates antigen presentation by DC to NKT cells and has been shown to protect again LPS mediated sepsis. ApoE self-derived peptides can induce monocytes differentiation into DCs and enhance T cell response.

This project has used an *in silico* methodology to predict potential biomarkers of DC vaccination responsiveness in tumour bearing patients. One new predicted potential molecule has been shown to be increased by conventional immunological methods validating this approach to biomarker discovery.

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The response to IL-7 re-emphasises the adaptive responsiveness of Il-17-producing gamma delta T cells

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Gamma/delta T cells include IFN-g-producing CD27+ (gd27+) and IL-17-producing CD27− (gd27−) subsets that are largely pre-programmed in the thymus. Since IL-17 provision by gamma/delta cells critically regulates IL-17 production and other inflammatory functions of alpha/beta T cells, it becomes important to elucidate factors respectively promoting gd27− *versus* gd27+ cell development and/or enrichment. Here we identify the cytokine, IL-7, as a significant regulator of IL-17-producing (gd27−) cells, both in the thymus and in lymph nodes, where the cells show evidence for constitutive exposure to IL-7. This is not the case for IFN-γ-producing (gd27+) cells. Thymocytes assume the distinctive phenotype (CD127$^+$, CD44hi, ICOShi) of lymph node (LN) IL-17-producing gamma/delta cells, being selectively enriched by IL-7. Thereafter, the full functional maturation of such gamma/delta cells can be realized by IL-7 and TCR-agonists in combination. These findings add substantively to an emerging role for IL-7-TCR collaboration in shaping T cell repertoires, have implications for rapid responsiveness within the lymphoid stress-surveillance compartment, and re-emphasise the potential adaptive responses of gamma delta cell subsets that are too commonly assigned to an 'innate-like' classification.

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IL-33 modulates the expression of human beta defensin-2 in human primary keratinocytes

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IL-33 is a member of the IL-1 family and has been implicated in allergic diseases such as atopic dermatitis and asthma. It is involved in the induction pro-allergic mediators in mast cells and basophils. The principal Th2 cytokine IL-4, found highly expressed in acute allergic eczema, is known to downregulate human beta defensin-2 (HBD-2) expression in human keratinocytes. A reduced production of HBD-2 has been linked to increased *Staphylococcus aureus* superinfection in patients suffering from atopic dermatitis. The purpose of this study was to investigate the effect of IL-33, which is associated with Th2 driven allergic inflammation on the expression of HBD-2 in human keratinocytes. HBD-2 expression by stimulated keratinocytes was measured by qRT-PCR and ELISA. Our results showed that TNFα and serum are both potent inducers of HBD-2 but the effect of serum was stronger. IL-33 significantly downregulated heat- inactivated serum-induced HBD-2 on the mRNA and protein levels. The downregulatory capacity of IL-33 was found to be 1.5 to two-fold weaker than IL-4. Interestingly, IL-33 failed to inhibit TNFα-induced HBD-2 expression. We have demonstrated the ability of serum to effectively induce HBD-2 and the ability of IL-33 to down-regulate the induced HBD-2. The signalling pathways underlying the contrasting effects of IL-33 on serum versus TNFα-induced HBD-2 needs to be elucidated in further studies. Our data suggest that IL-33 can significantly contribute to the decreased expression of HBD-2 in acute eczematous reactions clinically characterised by spongiosis and oozing - thus indicative for contact of the epidermis with serum components.

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IL-33 renders mast cells unresponsive to TLR ligands by inducing IRAK1 degradation

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Mast cells are located in tissues close to the environment and as such it is important that their response to TLR ligands is tightly regulated to avoid unnecessary activation. Here we show that pre-treatment of bone marrow derived mast cells with the epithelial cell product, IL-33, renders the cells insensitive to LPS and PGN. Responsiveness to antigen is retained and to some degree enhanced by pre-treatment with IL-33, suggesting that the effect is specific to the TLR/IL1R pathway. The inhibition of the LPS response is dependent on ST2, the IL-33 receptor, and is mediated by the degradation of the kinase IRAK1, which is part of the TLR/IL1R signalling pathway. IL-33 potently causes rapid IRAK1 degradation which is sustained for at least 24 h in mast cells, whilst the cytokine does not affect IRAK1 levels in macrophages. IRAK1 is known to have a redundant role in macrophage LPS signalling, but in contrast we show that it is required for TLR4 signalling in mast cells. LPS pre-treatment of mast cells also leads to IRAK1 degradation and insensitivity to further LPS treatment. We propose a hitherto unidentified role for IL-33 in maintaining immune homeostasis by retaining mast cells in an unresponsive state.

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CD4+ T cells, recruited via CXCL10 and CXCR3 interactions mediate chronic skin GVHD and distinguish acute from chronic disease post allogeneic stem cell transplantation

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Chronic Graft versus host disease (cGVHD) remains a major cause of morbidity and mortality following allogeneic stem cell transplantation (SCT) affecting up to 60% of patients who survive 100 days post transplant. Novel therapies for the treatment of cGVHD are required. As cGVHD exhibits tissue-specific pathologies the chemokine-receptor axis may present a potential therapeutic target for the treatment or management of disease. We therefore analysed a panel of chemokines in the serum of SCT patients with ($n = 40$) and without ($n = 18$) cGVHD. Chemokine receptor expression was then assessed in both peripheral blood and in fresh 4 mm skin punch biopsies using nine colour flow cytometry.

CXCL9, 10 and 11 were significantly associated with cGVHD of the oral mucosa, skin and eye, and with pulmonary cGVHD respectively. In particular CXCL10 was elevated fold−fold in chronic skin disease ($P < 0.05$). CD4 T cell expression of the CXCL10 specific receptor $CXCR3^+$ was reduced in the peripheral blood of patients with cGVHD from 23% of CD4+ T cells to just 5% ($P = 0.02$, $n = 5$), whilst CD4+, effector memory (EM), and CXCR3+ T cells were increased in the skin. Thus CD4+ EM CXCR3+ T cells are recruited to the skin during cGVHD. Elevation of CD4+ cells was not identified in patients with acute disease and distinguished acute from chronic disease. CXCL10 thus represents a specific chemokine which could be targeted to prevent or treat the symptoms of cGVHD of the skin, whilst CD4 tissue expression offers a novel method by which to diagnose acute from chronic disease.

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Innate and adaptive interleukin (IL)-17 responses provoked by chemical allergens: a comparison of two mouse strains

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The T helper (Th) 17 cytokine family, expressed by Th17 cells in adaptive immune responses, plays pivotal roles in autoimmune and allergic diseases. However, several innate sources have also been described, including $\gamma\delta$ T cells. We have evaluated the kinetics of chemical allergen-induced Th17 cytokine expression in BALB/c and C57Bl6 strain mice. Mice were exposed topically to the contact allergen 2,4-dinitrochlorobenzene (DNCB), the respiratory allergen trimellitic anhydride (TMA), or to vehicle alone. At selected time points single cell suspensions of draining lymph nodes were cultured and analysed for cytokine production and for phenotypic marker expression. A single exposure to either allergen resulted in transient up-regulation of Th17 cytokines in both strains. Maximal levels were observed at 6 and 48 h following exposure to DNCB and TMA, respectively. After repeated exposure under conditions where DNCB and TMA stimulate polarised Th1 and Th2 cytokine phenotypes, respectively, IL-17 production indicative of an adaptive immune response was recorded for DNCB-activated cells only. Interestingly, innate IL-17 levels were 30 times higher in BALB/c compared with C57Bl6 mice, and higher frequencies of $CD27^-$ $\gamma\delta$ T cells and of $CD27^+$ $\gamma\delta$ T cells were recorded in BALB/c and C57Bl6 mice, respectively. It has been reported previously that $CD27^-\gamma\delta$ T cells express preferentially IL-17 whereas $CD27^+\gamma\delta$ T cells secrete interferon-γ. These data suggest strongly that the lack of vigorous IL-17 production during the acute (innate) response in C57Bl6 mice (presumably elaborated by $\gamma\delta$ T cells) affects the subsequent adaptive Th17 response to chemical contact allergens.

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Toll-like receptor ligand-induced stimulation: comparisons of the XS106 skin dendritic cell (DC) line and bone marrow-derived DC

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Langerhans' cells (LC) are epidermal dendritic cells (DC). After encounter with antigen in the skin, LC, in addition to dermal DC (dDC), migrate to the lymph nodes and initiate an immune response. Toll like receptor (TLR) ligands were used to stimulate XS106 cells, a DC cell line isolated from murine epidermis, and bone marrow-derived DC (BMDC) from BALB/c strain mice. Surface expression of membrane markers MHC Class II, CD80, CD86 and CD40 on XS106 cells and BMDC was analysed by flow cytometry. Chemokine (type 1- [CXCL10] and type 2- [CCL17 and CCL22] associated) production was analysed by ELISA. Both BMDC and XS106 cells responded to type 2 TLR ligands peptidoglycan and flagellin by up-regulation of membrane marker expression. However, only BMDC up-regulated membrane marker expression after stimulation with CpG and R-848, TLR ligands that are associated with a type 1 phenotype. BMDC responded to type 1 and type 2 TLR ligands by up-regulating type 1- and type 2-associated chemokine production, respectively. In contrast, XS106 cells failed to up-regulate production of CXCL10 after stimulation with CpG or R-848. In addition, XS106 cells expressed constitutively high levels of type 2-associated chemokines, which was further enhanced by culture with peptidoglycan and flagellin. The TLR ligand response pattern demonstrates that XS106 cells display a preferential type 2 phenotype, similar to that reported for LC. Future experiments will address whether *in vivo* or *ex vivo* purified LC can respond to CpG and R-848.

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Platelet activating factor receptor expression in IL-23 stimulated cells

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Introduction: Platelet activating factor (PAF) mediates immune responses and its role has been implicated in autoimmunity. Our microarray studies revealed that PAF receptor was significantly upregulated by IL-23 but down regulated by IL-12. IL-12 is a pro inflammatory cytokine produced by antigen presenting cells and is recognized as the major inducer of Th1 cells that produce IFN-gamma. IL-23 is produced by activated macrophages and dendritic cells it supports the expansion of T cells that produce large amounts of IL-17.
Methods: Quantitative PCR was used to assess the expression of PAF-R on activated T blasts which were stimulated with IL-23. The expression of PAF-R on T-blasts was analyzed by flow cytometry. We investigated whether PAF-R on activated T blasts was functional by measuring the ability of cells to respond to PAF. Western blots were performed to observe if PAF activated JAK2 in T cells.
Results: The qpcr results revealed that PAF-R mRNA expression was unregulated by IL-23 and down regulated by IL-12. PAF-R expression was significantly higher on PBMC from Multiple Sclerosis patients in comparison to healthy controls. Intracellular calcium induction by PAF on T cells was enhanced by IL-23. Western blot analysis for JAK2 showed significant upregulation of JAK2 by PAF.
Conclusion: PAF-R is expressed as a functional receptor on activated T cells and this expression is enhanced by IL-23. Since IL-23 is important in Th17 cell development and expansion in future experiments we are aiming to reveal co-expression of PAF-R/IL-17 and investigate if IL-17 secretion is enhanced by PAF.

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The effect of platelets and placental microparticles on cytokine profiles during *in vitro* leucocyte culture for de novo anti-human platelet antigen (HPA)-1a production

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Placental syncytiotrophoblast microparticles (STMP) expressing fetal antigens, are shed throughout pregnancy into maternal blood. Neonatal alloimmune thrombocytopenia (NAIT) is due to maternal antibodies to fetal platelet antigens of paternal origin. Anti-HPA-1a, commonly implicated, targets a polymorphism on CD61, that is present on STMP and potentially responsible for primary immune responses in NAIT. To investigate this an *in vitro* culture system to generate *de novo* anti-HPA-1a is being developed.

In vitro immunisation of naïve HPA-1b1b, HLA-DRB3*0101 leucocytes with HPA-1a+ STMP and/or platelets was performed. A slow spin 'STMP' and an ultracentrifuged '100 g' preparation of placental vesicles were generated from term placenta. Monocyte derived dendritic cells were immunised with antigen, matured, then cultured with CD4+ and B/CD4− cells for 30 days. Culture supernatants were screened for 17 cytokines using multiplex bead immunobased assays analysed by Luminex and anti-HPA-1a by PIFT and/or MAIPA. Proliferation assays were performed to determine optimal antigen concentrations for immunization.

The most predominant cytokines were: IL-6,-8,-12,-13 and TGF-β with very low levels of IL-1b, -2, -5, -10 and IFN-γ. No IL-15, -17, -21 or IFN-α were detected. IL-4, GM-CSF and TNF-α were found at levels correlating with supplemented media. In all cases, increased levels of cytokines were seen with the addition of antigen with the exception of IL-13, where levels decreased. Hundred gram were the most stimulatory, followed by STMP and platelets. Platelets gave an equivalent or greater response for IL-6 and -8 yet caused the greatest level of suppression of IL-13. PBMC proliferation showed dose responses to antigen, with optimal stimulation at 40−60 mg/ml protein.

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IL-7 drives an early Th17 environment upon respiratory viral challenge

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IL-7 is a cytokine of great therapeutic potential as a treatment in chronic diseases due its ability to promote survival of both naïve and memory T cells. IL-7 therapy is well tolerated in primates with no detectable side effects and several clinical trials are underway to characterize the therapeutic benefits of IL-7 administration in HIV and cancer patients. However, little is known about its role in immunity to acute respiratory infections. Respiratory syncytial virus (RSV) bronchiolitis is the most common single cause of infantile hospitalization in the western world and we investigated the effect of IL-7 in RSV infection of mice. Responses to a recombinant RSV A2 strain engineered to express the murine *Il7* gene (RSV/IL-7) were compared to WT RSV A2. Challenge with the RSV/IL-7 vector enhanced both early (d2 p.c) and late (d7 p.c) pathology and viral clearance. The increase in early pathology is attributable to increased NK cell recruitment and activation, whereas increased CD4 T cell recruitment and activation is associated with later weight loss. Infection with RSV/IL-7 was also associated with an early Th17 response in the lung with increased IL-6, IL-17, IL-22, and MIP-3α production by $\gamma\delta$ and CD4 T cells. The Th17 response correlates with the presence of IL-7, as a Th1-biased response dominates by d7 p.c.

In conclusion, IL-7 boosts T-cell-mediated pathology during RSV infection by enhancing the innate and adaptive T cell response. This has significant implications for the use of IL-7 as part of a therapy or vaccine against respiratory infections.

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Suppressor of cytokine signaling 3 (SOCS3) enhances TLR5-induced TNFα expression in intestinal epithelial cells

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Inflammatory bowel disease (IBD), comprising Crohn's disease and ulcerative colitis, involves destruction of the epithelial lining of the intestine and increased pro-inflammatory cytokine production. SOCS3 is a negative regulator of pro-inflammatory cytokine (IL-6 and TNFα) signalling in intestinal epithelial cells (IEC), but IEC SOCS3 expression is enhanced in IBD. We investigated the effects of SOCS3 up-regulation upon IEC cytokine expression in response to microbial and inflammatory stimuli.

Stably transfected SW480 colonic epithelial cells over-expressing SOCS3, and controls, were used to assess cytokine expression following TLR3, TLR4, and TLR5 ligation using qPCR.

Flagellin (TLR5) and LPS (TLR4), but not Poly I:C (TLR3), treatment was shown to cause an increase in TNFα mRNA. When IEC over-expressed SOCS3, this resulted in enhanced TNFα expression in response to flagellin. All microbial ligands reduced the expression of TGFβ in both SOCS3 over-expressing and control IEC.

These studies suggest that increased expression of epithelial SOCS3 may be contributing to the increased production of pro-inflammatory TNFα associated with IBD.

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Role for IL-25 in hepatic and pulmonary fibrosis

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IL-25, a member of the IL-17 family, and its receptor IL-17BR are important in the genesis of type 2-medated responses to parasitic infection and allergic-like inflammation. Blocking IL-25 results in decreased inflammation in mouse models of asthma and colitis while administration of exogenous IL-25 can impair M1 and induce M2 macrophages in murine models of renal injury.

To assess the role of IL-25-mediated signalling in acute and chronic type 2 inflammation we used *Schistosoma mansoni* infection as a model. *Schistosoma mansoni* infection evokes a type-2 mediated granulomatous pathology in mice. WT, IL-25 and IL-17BR deficient mice were infected with *S. mansoni* and inflammatory responses monitored during acute (8 weeks) and chronic (14 weeks) stages of infection. Both IL-25$^{-/-}$ and IL-17BR$^{-/-}$ mice showed aberrant type 2 responses with smaller hepatic granulomas and attendant decreased collagen deposition. To dissect this further we used the synchronous schistosoma egg pulmonary granuloma model. Mice were injected with isolated *S. mansoni* eggs and granuloma formation in the lung analysed following primary and secondary, sensitized mice, challenge. IL-25$^{-/-}$ and IL-17BR$^{-/-}$ mice showed decreased granuloma size, accompanied by a reduced cellular infiltration and type 2 cytokines in the lungs as well as reduced pulmonary fibrosis. These data suggest a role for the IL-25 in fibrosis in both the lung and liver. Data on the underlying mechanism will be presented.

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RGS-1 in intestinal T cell trafficking and responsiveness

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Few clear mechanisms have been defined by which the regulation of gut T cell responses may be distinguished from systemic T cell regulation. In addition, how lymphocytes selectively respond to chemotactic signals in the gut is poorly understood. Regulator of G protein signalling-1 (RGS1) might be a key regulator of T cell trafficking in the gut based on its conspicuously high expression in gut-associated T cells. Our data shows that RGS1 is elevated in human and mouse gut T cells compared to peripheral T cells. RGS1 gain-of-function profoundly reduces T cell migration to lymphoid homing chemokines, whereas RGS1 depletion selectively enhances directional chemotaxis in gut T cells. RGS1 levels are further elevated in T cells derived from inflamed gut and the colitogenic potential of Rgs1-deficient T cells was significantly reduced in CD4^{+}CD45RB^{hi} T cell transfer model of colitis. Stimulation of gut T cells isolated from RAG2$^{-/-}$ mice injected with either Rgs1$^{-/-}$ or WT T cells induced similar cytokine production, suggesting that RGS1 represses T cell egress from the gut, possibly to sustain local immunoprotection and/or immunoregulation vis-à-vis commensals. Ongoing work aims to further discern to what degree RGS1 contributes to the unique state of rapidly responsive intraepithelial lymphocytes. In sum, RGS1 emerges as a novel, site-specific T cell regulator that may prove an effective clinical target, in particular in diseases where T cells become sequestered and with which RGS1 has been associated in genome wide association studies, namely celiac disease, multiple sclerosis and type I diabetes.

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IL-21 downregulates T cell IL-2 production and impairs Treg-mediated immune regulation

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Cytokines not only shape the compartment size of lymphocyte populations, but can also influence the effectiveness of Treg-mediated suppression. A clear example of this is provided by IL-21 that we have shown can potently counteract the ability of Treg to control conventional T cell responses. Accumulating evidence suggests a key role for IL-21 in autoimmune conditions such as type-1 diabetes; indeed IL-21 is upregulated in the DO11 x rip-mOVA mouse model of autoimmune diabetes and ablation of IL-21 signalling in NOD mice is known to protect from diabetes. The ability of IL-21 to counteract Treg suppression may represent one mechanism by which this cytokine promotes autoimmunity. To further explore this phenomenon, we have dissected which cell type is the target for IL-21 during the release from Treg-mediated suppression. We show that IL-21 counteracts Treg suppression by acting on conventional T cells and that this is associated with inhibition of IL-2 production. Despite IL-2 deprivation, conventional T cell responses proceed unimpaired since IL-21 can substitute for IL-2 as a T cell growth factor. However IL-21 fails to substitute for IL-2 in maintaining the Treg population. Thus IL-21 signaling in conventional T cells alters Treg homeostasis by decreasing IL-2 availability. These data demonstrate that IL-21 and IL-2 can have overlapping roles in promoting conventional T cell responses but play distinct roles in controlling Treg homeostasis and function.

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Induction of Interleukin-10 expression by the commensal flora

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Interleukin-10 (IL-10), a pleiotropic cytokine, is a crucial anti-inflammatory mediator playing a non-redundant role in the intestine. The importance of this cytokine has been illustrated by knock-out mice which develop a lethal colitis in response to the normal colonic flora. Recent studies have shown that IL-10 is predominantly expressed by $Foxp3^+$ regulatory T-cells in the colonic lamina propria.

In this study we aimed at further characterizing the subset of IL-10 secreting $Foxp3^+$ T-cells and at dissecting the mechanisms governing their activation in the intestine.

Therefore we employed a novel double reporter mouse which monitors IL-10 expression by co-expression of GFP and Foxp3 expression by co-expression of a non-functional human CD2 protein. We and others could show that the induction of IL-10 expression ulitimately depends on the commensal flora, as treatment with broad spectrum antibiotics abrogated IL-10 expression. Conversely colonization with *Helicobacter hepaticus* significantly enhanced the frequency of $Foxp3^+IL\text{-}10^+$ cells in the colonic lamina propria. We could show that under steady-state conditions induction of IL-10 expression did not depend on IL-2, IL-12, IL-15, IL-21 and IL-10 signaling itself. Using TCR V_b-chain spectrotyping we show that $IL\text{-}10^+$ $Foxp3^+$ cells represent a broad array of specificities, this analysis did as well not show an antigen specific skew after infection of the mice with *Helicobacter hepaticus*.

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Aberrant basal and TLR-stimulated expression of TSLP in rheumatoid synovial fibroblasts

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Background and aims: Thymic stromal lymphopoietin (TSLP) is an interleukin-7-like cytokine, which strongly activated dendritic cells for Th2 polarization. Previous collagen-induced arthritis model demonstrated its role in exacerbating disease severity via T-cell dependent mechanism. Here we investigated TSLP and its receptor (TSLPR) expression in the synovium of rheumatoid arthritis (RA) patients and in rheumatoid synovial fibroblasts (RASF), in basal conditions and upon stimulation with Toll-like receptors (TLR) ligands.
Methods: mRNA and protein (cytoplasmic and soluble) expression of TSLP in RASF, osteoarthritis (OASF) and RA dermal fibroblasts (RADF), stimulated with or without TLR2, TLR3 and TLR4 ligands, was assessed by Taqman PCR (QT-PCR), immunocytochemistry and ELISA. TSLP and TSLPR expression in 40 synovium of RA patients was investigated by QT-PCR and immunohistochemistry.
Results: RASF and, to a lesser extent OASF, constitutively displayed higher TSLP mRNA (approximately 8–16-fold) compared to RADF. *In vitro* stimulation of TLR3 and TLR4, but not TLR2 on RASF led to strong induction of TSLP mRNA expression (approximately 20-fold increase with TLR3), which peaked early at 8 h. Cytoplasmic staining of TSLP was increased in TLR3-activated RASF but not RADF, while soluble TSLP was time-dependently released in the supernatant of TLR3-stimulated RASF (approximately 100 pg/ml) and undetectable in RADF. TSLP mRNA was observed in all the RA samples examined while TSLPR was significantly increased in patients with follicular synovitis.
Conclusions: Overall, our data strongly support a pivotal role for RASF in the dysregulated production of pro-arthritogenic/inflammatory TSLP in the rheumatoid synovium, suggesting that the TSLP/TSLPR pathway contributes to chronic inflammation in RA.

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The role of monoclonal antibody affinity in mediating protection against autoimmune inflammatory diseases

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Monoclonal antibodies (mAbs) have recently emerged as new drug modalities for the treatment of chronic inflammation. Indeed, blocking cytokines using mAbs is an established disease-modifying therapy for inflammatory diseases including rheumatoid arthritis. It is generally assumed that mAbs need to have a high affinity for the target cytokine in order to show efficacy. However, no conclusive studies have been conducted which directly address this issue. To elucidate this question, we generated a panel of mouse mAbs specific for Interleukin 17 (IL-17), all of which potently neutralized the effects of this key mediator of autoimmune inflammatory disorders *in vitro*. The variable regions of a selected hypermutated high affinity anti-IL-17 antibody differed in three amino acid residues compared to the likely progenitor. Back mutation to germline sequence resulted in a 100-fold reduced affinity for IL-17. The ability of these two antibodies, which recognize the same epitope with different affinities, to block chronic inflammation was subsequently tested in murine models of autoimmunity. The parental lead antibody as well as the derived germline antibody were able to delay disease onset and significantly reduced disease severity. These results indicate that the affinity of germline cytokine specific antibodies may be sufficient for protection against autoimmune inflammatory diseases.

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Interleukin 33 induces CD4 Th2 and nuocyte effector responses via the activation of mTOR signalling pathways

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IL-33 is a member of the IL-1 cytokine family that promotes the effector function of CD4 Th2 cells and innate immune cells including the recently described innate helper cell / nuocyte population. IL-33 mediates its biological effects via interaction with a specific receptor ST2 and has been implicated in inflammatory pathologies including asthma, anaphylaxis and autoimmunity. Therefore, an understanding of the signalling pathways that regulate IL-33-dependent responses may suggest new therapeutic approaches to combat inflammation.

The mammalian target of rapamycin (mTOR) signalling pathway regulates cell growth and proliferation and is inhibited by the immunosuppressant macrolide rapamycin. We sought to determine the role of mTOR in IL-33-dependent responses. IL-33 directly induced mTOR activation in an ST2-dependent manner in CD4 Th2 cells and lung-derived nuocytes. Furthermore, IL-33-dependent cytokine secretion (IL-5 and IL-13) by Th2 cells *in vitro* and nuocytes *in vitro* and *in vivo* was inhibited by rapamycin. Furthermore, airway inflammation induced by intranasal inoculation of mice with IL-33 was reduced by concomitant administration of rapamycin. Together these data suggest an important role for mTOR signalling in the biological effects of IL-33 mediated by both CD4 Th2 cells and nuocytes *in vivo*.

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Virtual screening identifies a biologically active novel family of CB2R selective compounds (with improved physicochemical properties)

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The endogenous endocannabinoid system modulates inflammation and immune responses. It consists of two G-protein-coupled receptors, CB1R expressed in the central nervous system and CB2R expressed in leukocytes. Activation of CB1R, but not CB2R, has psychoactive effects.
Aim: Identify novel selective CB2R ligands using virtual screening and assess the effect of these novel CB2R agonists on macrophage CB2R responses.
Methods: Conformers of a known selective CB2R agonist were screened against 1 932 300 conformers of the MedChem library using the programme ROCS. The screen's hits were functionally assessed in a hCB2R cAMP assay.
Results: A novel class of compounds was identified with submicromolar CB2R activity. hCB1R/hCB2R affinity studies demonstrated more than 10 compounds from this family are at least 1000-fold selective for CB2R over CB1R. These compounds are being tested in functional assays with primary murine macrophages and in *in-vivo* inflammation models.

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IL-10 production by apoptotic tumor cell-activated macrophages requires signaling through TrkA - validation of an adenoviral RNAi screen

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Tumor-associated macrophages (TAM) are a major supportive component within neoplasms. Mechanisms of macrophage attraction and differentiation to a tumor-promoting phenotype, characterized by pronounced interleukin (IL-10) production, are ill-defined. Here we aimed to identify signalling pathways that contribute to the generation of TAM-like macrophages using an RNAi approach. Primary human monocyte-derived macrophages were stimulated with apoptotic tumor cell supernatants (ACM), which induce a TAM-like phenotype in human macrophages characterized by production of IL-10, IL-6, IL-8 and repression of IL-12. Beforehand, macrophages were transduced with the adenoviral shRNA SilenceSelect® library of GalapagosBV, which aims at potential drug targets, and release of IL-10, IL-6, IL-8 and IL-12 was determined. We identified 108 gene involved in IL-10 production in response to ACM and observed a pronounced cluster of targets regulating both IL-10 and IL-6. We validated the most promising target in detail, which is the nerve growth factor (NGF) receptor TrkA, whose role in immunity is slowly emerging. Mechanistically, sphingosine-1-phosphate (S1P) release from apoptotic cells triggered src-dependent shuttling of cytosolic TrkA to the plasma membrane via S1P receptors. Plasma membrane-associated TrkA, which was activated by constitutively autocrine secreted NGF, triggered PI3K/AKT and p38 MAPK signaling to induce IL-10. Interestingly, TrkA-dependent signaling was also required for cytokine production by myeloid cells isolated from primary murine breast cancer tissue, indicating relevance in cancer-associated inflammation. Our findings highlight a fine-tuned regulatory system including S1P-dependent TRKA trafficking for executing TAM-like cell function *in vitro* as well as *in vivo*.

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Natural killer cells, cytotoxic and intraepithelial lymphocytes depend for their development, maturation and functionality on different levels of Interleukin-15 expression as well as cellular sources

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Aims: The c-gamma-chain cytokine interleukin (IL)-15 is known to facilitate the maturation and survival of natural killer (NK) cells, cytotoxic T cells (CTLs) and gut intraepithelial lymphocytes (IELs). Apart from stromal cells, CD11c+ dendritic cells have been shown to express high levels of IL-15. The aim of our study was to assess the specific and differential contribution of CD11c+ cells to the IL-15-mediated effect on target cell populations.
Methods: We analyzed the phenotype and immune status in a newly generated set of transgenic mouse lines, in which IL-15 expression originates exclusively from a CD11c-driven transgene. Importantly, the transgenic IL-15 expression occurs at different levels which allowed the correlation of IL-15 levels with different developmental and functional maturation events in the target populations.
Results: Memory CTLs require low levels of CD11c-driven IL-15 for full maturation and expansion. Differently, splenic NK cells displayed a fully mature phenotype and increased frequencies only upon IL-15 overexpression. Yet, the IEL populations remained absent or strongly reduced in all mouse lines.
Conclusions: IL-15 expressed by CD11c+ cells contributes to the maturation and survival of several IL-15 dependent populations. However, target populations require a specific level of IL-15 expression with NK cells demanding higher levels than CTLs. Of note, both populations could be fully reconstituted in numbers, even though IL-15 expression was genetically restricted to CD11c+ cells. In turn, the IEL populations in the gut seem to be dependent on IL-15 from other sources, since even very high levels of CD11c-derived IL-15 could not support their survival.

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Using intravital microscopy to investigate the molecules that regulate the homing of senescent neutrophils to the bone marrow and their phagocytosis by bone marrow macrophages

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Neutrophils are released from the bone marrow into the blood, where they have a half-life of about 6.5 h in man. As they age, circulating neutrophils up-regulate their cell surface expression of CXCR4 and home back to the bone marrow where the CXCR4 ligand, CXCL12 is constitutively produced by bone marrow stromal cells. Although the biological significance of this process is not clear, it has been shown that aged neutrophils are phagocytosed by bone marrow macrophages, suggesting a novel route for the clearance of apoptotic neutrophils during homeostasis. This project aims to develop a methodology to visualize the homing of senescent neutrophils to the bone marrow in real time, *in vivo*, using intravital (IVM) microscopy of the mouse calvarium bone marrow. Initial IVM experiments revealed that labelled, circulating neutrophils can be seen moving in blood vessels of the calvarium bone marrow 30 min after injection and that, 24 h after injection, senescent neutrophils 'cluster' in specific areas of the bone marrow. This data suggests that there are specific anatomical regions in the bone marrow that support neutrophil homing to the bone marrow.

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Interleukin 7 signaling on DCs regulates the CD 4 T cell niche

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IL-7 is a homeostatic cytokine produced mostly by nonhematopoietic cells which signals on both T cells and DCs. We are studying the role of IL-7 signaling on DCs in self-tolerance and T cell homeostasis. We hypothesize that IL-7 signaling on DCs is important for T cell homeostasis and plays a central role in maintaining immune tolerance to gut antigens. We predict that interruption of IL-7 signaling on DCs will increase the incidence and/or severity of autoimmune colitis.

CD103+ DCs harvested from the MLN and the lamina propria of normal mice have been shown to be tolerogenic, promoting the differentiation and proliferation of Tregs. $CD103^+$ versus CD103− DCs harvested from the MLN of normal mice were analyzed for expression of IL-7R. IL-7R gene expression was assayed by quantitative PCR normalized relative to expression of HPRT. Tolerogenic $CD103^+$ DCs showed a 10-fold increase in the expression of IL-7R.

To determine if lymphopenic mice with deficient IL-7Ra signaling on DCs have increased proliferation of CD4+ T cells and if there is a change in the size of the Treg population, we created IL7R−/−Rag1−/-. We confirmed enhanced homeostatic proliferation of CD4+ T cells in mice with deficient IL-7 signaling when compared to Rag1−/− controls. The size of the Treg population increased proportionally to the size of the CD4 T cell pool.

These results confirm that IL-7R+ DCs are regulators of the peripheral CD4+ T cell niche. Future studies include using this axis in a murine model of T cell transfer colitis.

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Expression IP-10 and CXCR3 transcripts in peripheral blood of ovarian cancer patients compared to control cases

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Ovarian cancer has the highest mortality rate among gynecologic cancers. The high case fatality rate is partly the result of delayed diagnosis and the lack of an effective treatment for women who have advanced disease.

To further examine the importance role of CXCR3 and CXCL10/IP-10 in the cancer patients, we will evaluate the expression levels of CXCR3 and CXCL10/IP-10 transcripts in the peripheral blood ovarian cancer patients by Real Time PCR (RT-PCR) method in compare healthy women and also their correlation with each other will be examined.

Peripheral blood specimen from 48 patients with ovarian cancer and healthy women with same age was collected. Total RNA was extracted with TRIzol reagent and cDNA was synthesized. Expression of β-actin (housekeeping gene), CXCR3 and IP-10 was evaluated using Real-Time PCR and Syber green I as reporter dye. At the final step of each run, Melting Curve analyzing was performed to confirm validity of results. Statistical analyzing was done using SPSS software by Mann−Whitney and correlation tests.

As a result the expression level of CXCR3 was not significantly different in patients when compared with control group. However, IP-10 expression increased significantly in ovarian cancer patients in comparison with control cases ($P < 0.05$). Moreover, IP-10 expression was significantly correlated with CXCR3 expression in both patient and control groups ($P < 0.001$).

Results of this study showed that expression level of some gene such as IP-10 can be applied in cancer diagnosis but validity and accuracy of this finding must be further examined in extended groups.

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Molecular links between inflammation and brain function

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Patients with chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease or psoriasis are often further burdened with neuropsychiatric symptoms such as depression, anxiety and fatigue. Despite the recent advances in our understanding of neuroimmune communication pathways, the molecular mechanisms behind these co-morbidities remain unclear. Utilising transcriptomics in a well-characterised animal model of systemic inflammation, we have started to investigate the molecular mechanisms by which inflammation originating in the periphery can induce neurological transcriptional modulation and resulting behavioural changes.

Inflammation was induced in male C57BL6 mice via intraperitoneal injection of lipopolysaccharide (LPS). The transcriptional profile of the brains of these mice was compared to that of a vehicle-injected control group using microarray analyses. We demonstrated that LPS-induced inflammation triggers an increase in transcription of a range of proinflammatory molecules in the central nervous system (CNS); many of which are regulated by type I interferons. This transcriptional response is indicative of peripherally triggered, interferon-mediated, CNS inflammation. There is now significant literature supporting the link between type I interferons and psychiatric disorders. Consequently, interferon production in the CNS may be a potential mechanism linking peripheral inflammation with behavioural alterations.

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CCR5 mobility at the surface of human macrophages and localisation upon CD4+ T cell engagement

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The chemokine receptor CCR5 is highly expressed on macrophages and is thought to help direct these phagocytes to sites of inflammation where antigens are engulfed then processed peptides presented in the context of MHC-II to armed CD4+ T cells. In light of recent findings that in human T cells CCR5 becomes localised to the interface of the T cell- B cell immunological synapse, the present study investigates the behaviour of CCR5 during the formation of the macrophage- CD4+T cell immunological synapse. The mechanism of CCR5 recruitment to the immunological synapse likely involves lateral movement of the receptor within the plasma membrane, as is the case for many other receptors organised into spatially segregated domains at the immunological synapse. For this reason the mobility of CCR5 in the macrophage plasma membrane was assessed by FRAP. Initial results indicate the presence of an immobile fraction of plasma membrane CCR5 in both macrophage and CCR5-transfected CHO cells. Confocal observation of fixed macrophage- CD4+ T cell conjugates shows that CCR5 is localised to the cell-cell interface in a discrete manner. In addition, a flow cytometry protocol was developed to validate macrophage- CD4+ T cell immunological synapse formation, enabling analysis of the number of conjugates formed and any intracellular calcium signalling that results from conjugate formation with or without superantigen. Together these studies will shed further light on how CCR5 becomes discretely localised to the macrophage- CD4+ T cell immunological synapse.

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The effect of native-LDL and oxidized-LDL in altering immune responses in atherosclerotic patients

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Oxidized and native low-density lipoproteins (ox-LDL, n-LDL) are instrumental factors in atherogenesis; however, their effect on immunoregulation in stable plaques of coronary syndrome is still unclear. CD4+CD25+FoxP3+ regulatory T (Treg) cells and Th17 cells, a subset of T-helper cells, play an important role in peripheral immunity. Their imbalance leads to the development of tissue inflammation and autoimmune diseases. A few studies have explored the effect of ox-LDL and n-LDL on the balance between T-reg and Th17. In this study, peripheral blood mononuclear cells (PBMCs) from patients with stable angina (SA) and individuals with normal coronary artery (NCA) was used to investigate the effect of n-LDL and ox-LDL on the frequencies of Treg cells, and the levels of interleukin-10 (IL-10), interleukin-17 (IL-17) and interleukin-6 (IL-6). Our results demonstrated that SA patients have shown a significant decrease of Treg frequency, and cell culture supernatant Interleukin-10, whereas both SA and NCA have shown an obvious decline of Interleukin-6 secretion levels, known as Th17 inducing cytokine.

We suggest that this maintained tolerant effect will be altered under the effect of a cytokine milieu that can shift the immune system either to an inflammatory or anti-inflammatory response depending on the cytokine nature and lipoproteins concentration in the medium.

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Novel sources of IL-9 in lung inflammation

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Interleukin-9 (IL-9) is a cytokine implicated in lung inflammation with CD4 T cells reported to be the major cellular source of abundant IL-9 production, but the exact mechanism of its function remains unclear. We generated a reporter mouse strain designed to fate map cells that have activated IL-9. During papain, but not ova-induced lung inflammation IL-9 production was largely restricted to innate lymphoid cells (ILC) instead of CD4 T cells. The fate reporter identified plasticity and rapid loss of IL-9 protein production in favor of other cytokines such as IL-13 and IL-5. IL-9 production was dependent on adaptive immune cells and blockade of IL-9 production via neutralizing antibodies substantially reduced IL-13 and IL-5, suggesting that ILC could provide the missing link between the well-established functions of IL-9 on the regulation of TH2 cytokines and responses.

Cellular Interactions

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Inhibition of long-term kindled seizures induced alterations in hematopoietic functions in Bone marrow cells by AC-31B (Essential oil from *Allium cepa)*

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Epilepsy is a condition characterized by recurrent seizures due to abnormal excitability of nerve cells. Many available antiepileptic drugs can result in the disruption of hematopoietic functions thereby alteration in the immune function. Any change in the bone marrow (BM) which provides a suitable environment for proliferation and differentiation of hematopoietic stem cells, could impact on the development of the components of immune system. The present study sought to determine whether changes in hematopoietic functions following kindling are likely to be controlled by anticonvulsants isolated from natural products. The markers of hematopoietic cells used for this study were CD29, CD44 and CD90. The immunostaining of cultured BMSCs revealed the highest expression of these markers in BM progenitor cells and colony numbers in CFU-GM cultures following long-term PTZ-induced kindled seizures in mice receiving no other treatment. Amongst the treatment groups, the diazepam treatment demonstrated high expression of CD29, CD44 and CD90 comparable to that of the PTZ- kindled control animals. In contrast, reduced expression of CD29, CD44 and CD90 was observed in AC-31B (essential oil from *Allium cepa*) treated group. Our data indicates that there is immune response magnification in PTZ-kindled control and diazepam treated groups. However, the suppression of kindling along with moderate expression of the CD markers on the cells of AC-31B treated group suggests the presence of potent anti-epileptogenic compound(s). The further studies on isolation of AC-31B oil may lead to discovery of a naturally occurring anticonvulsant drugs with no or mild effects on the hematopoeitic functions

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Dendritic cell transfected with mRNA from colon cancer cells pretreated with 5-fluorouracil decrease the tumor growth in murine model

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We recently observed that human DCs treated with low doses of antitumor chemotherapeutic agents became them more efficient to stimulate T lymphocytes. On the other hand, treatment of tumor cells with such low concentrations of selected drugs made them more immunogenic than wild type cells. In the present study we aimed to evaluate (i) whether transfection of DC with drug-treated tumor cells RNA, enhances the effectiveness of DC-based vaccine, and (ii) if the modulatory effects of chemotherapeutics can be observed *in vivo*. To achieve these goals, C57/Bl-6 mice were subcutaneously inoculated with MC-38 cells and 10 days later they were treated with DC sensitized with RNA from tumor cells pre-treated with a minimum effective concentration (MEC) of 5-fluorouracil. Vaccination with mRNA-transfected DC significantly decreased the tumor growth, being the tumor size 40% lower than the Control group. Analysis of DC phenotype showed that transfection increased the percentage of CD86 (55% higher than control), CD40 (57% higher), and MHC class II-expressing cells (58% higher). In order to analyze the specific immuneresponsiveness of tumor-bearing mice, spleen cells were co-cultured with MC-38 target cells and the supernatants evaluated on the levels of IFN-g. Results have shown that vaccination with DC was able to increase the *in vitro* production of this cytokine. These results indicate that treatment of tumor cells with 5-FU induces transcriptional changes that can be transfered to DC by RNA transfection, enhancing their ability to stimulate the antitumor response. Financial support: FAPESP 2009/18331-8; FAPESP 2010/06013-9.

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The effects of T_{h17} cytokines on liver parenchymal cells shape the microenvironment for local generation of T_{h17}/T_{c17} in inflammatory liver disease

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Background: IL-17 secreting T cells have been implicated in autoimmunity, inflammatory disease and provide a link between the innate and adaptive immune responses. High numbers of IL-17-producing T cells which also secrete IL-21 and IL-22 are found in close proximity to bile ducts in several liver diseases. T_{h17} related cytokines have multiple effects and may be involved in both effector responses and repair and regeneration.
Methods: Primary human parenchymal cells were assessed for cytokine receptor expression. The effects of stimulation with recombinant IL-17, IL-21, IL-22, TNFα or IFN-γ alone or in combination were compared for apoptosis using annexin staining, proliferation was measured by *in situ* Ki67 staining and secretion of IL-1β, IL-6, IL-23 and TGF-β1 was assessed by ELISA.
Results: All parenchymal cells expressed IL-17R, IL-21R and IL-22R. T_{h17} cytokines did not cause apoptosis but led to parenchymal cell proliferation. Cholangiocytes and hepatocytes responded best to IL-17, whereas sinusoidal endothelial cells were responsive to IL-22. Cholangiocytes responded to T_{h17} cytokines by secreting high levels of IL-1β, IL-6, IL-23 and TGF-β1 all cytokines that support the survival of T_{h17} and T_{c17} cells.
Conclusions: Liver parenchymal cells express IL-17, IL-21 and IL-22 receptors and proliferate in response to T_{h17} cytokines. Cholangiocytes also respond by secreting T_{h17}/T_{c17} polarising cytokines. Therefore T_{h17} related cytokines secreted by infiltrating lymphocytes may activate the epitheliome to generate a local environment characterized by cholangiocyte proliferation and T_{h17} / T_{c17} cell survival, thus contributing to bile duct proliferation and persistent chronic inflammation that characterizes many liver diseases.

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A role for the pattern recognition receptor Nod2 in promoting recruitment of CD103$^+$ DC to the gut in response to *Trichuris muris* infection

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The ability of the colon to generate an immune response to pathogens, such as the whipworm *Trichuris muris,* is a fundamental and critical defence mechanism. Our previous work demonstrated that resistance to infection is associated with the rapid recruitment of dendritic cells (DCs) to the colonic epithelium via epithelial production of CCL5 and CCL20. However, the epithelial-parasite interaction that drives chemokine production is not known. Here, we address the role of the cytosolic pattern recognition receptor Nod2, the location of which within the crypts correlates with the *T. muris* niche. *Nod2*$^{-/-}$ mice have a delayed expulsion of *T. muris.* In WT mice there was a rapid influx of CD103$^+$CD11c$^+$ DCs into the colonic epithelium, whereas, this recruitment was impaired in *Nod2*$^{-/-}$ animals. Strikingly, the number of colonic CD11c$^+$CD103$^+$ DCs in Nod2$^{-/-}$ mice remained low until D7 post-infection. Migration assays revealed no difference between the migration of *Nod2*$^{-/-}$ and WT colonic DCs in response to chemokines. However, *in vivo and in vitro* experiments show epithelial production of chemokines, CCL2, CCL3 and CCL5 by *Nod2*$^{-/-}$ epithelial cells to be markedly reduced. Furthermore, bone marrow chimeras of wildtype mice reconstituted with *Nod2*$^{-/-}$ cells equivocally demonstrated that *Nod2*$^{-/-}$ DC recruitment to the epithelium was normal in response to *T. muris*. Collectively, these data suggest a role for Nod2 in mediating epithelial chemokine production in the response to *T. muris* and recruitment of CD103$^+$ DCs to the colonic epithelium.

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Immunomodulatory properties of MSCs on Th17 cells: the role of cell-contact and IL-6

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Mesenchymal stem cells (MSC) are multipotent stem cells with broad immunosuppressive properties over the innate and adaptive immune system. However, we have found MSCs to suppress Th1 cells while promoting Th17 cell development once target T cells have been activated. We therefore sought to evaluate the role of soluble factors, -including IL-6- as opposed to direct cell contact with regard to the immunoregulatory effects of MSCs on CD4$^+$ T cell subpopulations after early (day 0) or late (day 2 and 4) T cell activation. T-CD4 cells were obtained from mice splenocytes, differentiated into Th1 or Th17 cells and cultured with MSCs. After 6 days of coculture, the production of intracellular cytokines (IL-17A and IFN-g) and transcription factors (rorgt and tbet) were measured by flow cytometry and real time PCR respectively. Upon early T cell activation, MSCs achieved Th1 as well as Th17 suppression irrespective of cell contact. However once cell activation has occurred, MSCs were able to exert a 50% inhibition on Th17 cells only with cell-contact ($P < 0.05$) while not suppressing without cell contact. Of interest, when testing IL-6 deficient MSC we observed a partially recovering in the immunosuppressive properties of MSCs in a 40% ($P < 0.05$) on Th17 cells. Altogether, these data suggest that the immunosuppressive properties of MSCs on Th17 cells are dependent on cell-contact and in this context; IL-6 expression by MSC may have a role maintaining the Th17 phenotype, but only on later states of T-cell activation.

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The molecular mechanisms of B cell and B cell lymphoma recruitment to the human liver

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B cells within liver tissue have been shown to contribute to chronic inflammation but the recruitment signals for B cells are poorly understood. B cell lymphomas also infiltrate the liver but again little is understood of the recruitment mechanisms. Recruitment occurs within low shear vascular beds which are lined by specialised hepatic sinusoidal endothelial cells (HSEC). Our aim was to understand the mechanisms of B cell and B cell lymphoma recruitment to the liver.

We used isolated human HSEC in flow assays with B cells and two B cell lymphoma cell lines. We measured adhesion, transmigration, direction and velocity of cell migration through endothelium under flow. The contribution of adhesion molecules was studied by using function blocking antibodies and the effect of specific chemokines by adding them to the endothelial cells.

The primary adhesion receptor for B cells on HSEC was VCAM-1. Compared with T cells fewer B cells underwent transendothelial migration and they showed restricted migratory activity on endothelium under flow. B cell migration was mediated by ICAM-1, VAP-1 and CLEVER-1/stabilin-1. Lymphoma cell line recruitment shared several features of primary lymphocyte homing, firm adhesion was mediated by ICAM-1 and VCAM-1 and they demonstrated crawling behaviour which was ICAM-1 dependent. However the lymphoma cell lines did not undergo transendothelial migration.

The recruitment signals we have identified for B cells in this study may provide potential therapeutic targets for liver disease. Preserved lymphocyte homing mechanisms in malignantly transformed B cells could be therapeutic targets to prevent lymphoma dissemination to the liver.

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Direct transcutaneous targeting of antigen to the specific skin dendritic cell subsets induces antigen specific CD4+ and CD8+ T cell responses

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Dendritic cells (DC), key professional antigen presenting cells, play a pivotal role in linking the innate and adaptive immunity. Recently, several studies highlighted the complexity and functional specialization of the cutaneous DC network, showing that at least five subsets of DC coexist in murine skin and that these DC subsets may exhibit specific immune functions. Therefore, due to the high number of DC present in the epidermis and dermis, the skin represents an optimum site for vaccine delivery. For the first time dissolvable polymeric microneedle (MN) arrays laden with ovalbumin (OVA) encapsulated nanoparticles (NP) were explored to target skin DC subsets to promote enhanced immune responses. We demonstrated that following the efficient internalization of fluorescent OVA NP, bone marrow derived DC up-regulated maturation markers expression and induced OVA specific $CD4^+$ and $CD8^+$ T cell proliferation *in vitro*. Following application of MN laden with rhodamine encapsulated NP, we confirmed efficient skin delivery of NP in situ and demonstrated that skin DC were able to successfully uptake and deliver fluorescent NP to cutaneous draining lymph nodes. Furthermore, following the application of MN loaded with OVA NP, ex-vivo purified DC from cutaneous draining LN induced proliferation of OVA specific, IFN-g producing effector $CD8^+$ T cells. Finally, we confirmed that skin DC subsets promoted *in vivo* proliferation of OVA specific, adoptively transferred $CD8^+$ and $CD4^+$ T cells. Therefore, direct antigen targeting to the specific skin DC subsets will help us understand the precise contribution of particular skin DC subsets during antigen specific immune responses.

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$CD11c^+$ lung antigen presenting cells (APCs) from RSV infected mice have an enhanced ability to induce Th2, but not Th1, recall responses to allergen

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Respiratory syncytial virus (RSV)-bronchiolitis during infancy is associated with an increased risk of childhood asthma. Healthy neonates often already display T-helper (Th)-2 cell responses to allergen. RSV-infection may therefore re-enforce pre-existing, asymptomatic, allergic sensitisation increasing the potential for asthma development.

Here, we examined in a murine model whether $CD11c^+$ lung APCs following RSV-infection have an increased capacity to induce recall Th2-responses to allergen. $CD11c^+$ APCs isolated from lungs of mice infected with RSV (RSV-APCs) or UV-inactivated RSV (UV-APCs) were co-cultured in the presence of ovalbumin (OVA) with $CD4^+$ T-cells, previously isolated from the spleen of DO11.10 mice and polarised *in vitro* to Th1- or Th2-cells. After 72 h T-cell proliferation and cytokine production were analysed.

Th2-cell proliferation at low concentrations of OVA was found to be significantly increased in co-cultures with RSV-APCs compared to UV-APCs. Th1-cell proliferation was observed only with high OVA concentrations in co-cultures with UV-APCs but not RSV-APCs. Co-culture with RSV-APCs resulted in increased production of Th2-cytokines by Th2-cells and interestingly, in the case of IL-4 and IL-13, also by Th1 cells which also produced IFN-gamma.

The increased ability of lung APC following RSV infection to induce Th2-cell recall responses may facilitate subsequent development of allergic airways disease and in infants may augment previous low level allergen sensitisation with an increased risk of asthma development.

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Probiotics modulate epithelial cell barrier properties influenced by co-culture with macrophages

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Gut macrophage-derived cytokines are instrumental to mucosal immune responses: tolerance or inflammation. Dependent on prevalence of immune mechanisms, probiotic bacteria may modulate these macrophages, exerting either beneficial effects by enhancing intestinal barrier function or detrimental; perpetuating inflammatory destruction of epithelial barrier and underlying mucosal tissue. The aim of this study was to investigate modulation of macrophage-influenced epithelial barrier function by probiotic bacteria. Using a transwell co-culture system, transwell Caco-2 epithelial cells were incubated in the presence of basolateral M1-like (pro-inflammatory) and M2-like (regulatory) macrophage subsets, apically applied probiotics and inflammatory stimuli (IL-1b and LPS). Parameters investigated included transepithelial electrical resistance (TEER) and gene expression, immunohistochemical staining of the tight junction protein, zona occludin-1 (ZO-1). TEER and ZO-1 expression were down-regulated upon co-culture with pro-inflammatory M1 macrophages. In the presence of LPS, *L. fermentum* enhanced TEER and ZO-1 whereas *L. casei* Shirota (LcS) had no effect. In contrast, in the presence of IL-1b, LcS and *L. fermentum* down-regulated TEER and ZO-1. In the presence of M2 regulatory macrophages, both probiotics enhanced TEER in the presence of LPS yet decreased TEER with IL-1b. In conclusion, probiotic modulation of mucosal barrier properties is determined by strain, inflammatory environment and mucosal macrophage effector phenotype.

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Pharmacological inhibition of glycogen synthase kinase 3 regulates T cell development *in vitro*

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The development of functional non-autoreactive T cells requires receptor-mediated transition through multiple checkpoints in the thymus. Double negative 3 (DN3) thymocytes are selected for the presence of a rearranged TCR beta chain in a process termed beta-selection which requires signalling via the preTCR and Notch1. Pre-selection DN3 are referred to as DN3a and express low levels of CD27 and CD98, while post-selection DN3 (which are TCR beta positive) express higher levels of CD27 and CD98. Additional signalling from Stromal-Derived Factor 1alpha (SDF1alpha)/CXCL12 ensures optimal proliferative expansion of DN3 thymocytes. Signal integration by these receptors converges on core pathways such as the Phosphatidylinositol-3-kinase (PI3K) pathway. Glycogen Synthase Kinase 3beta (GSK3beta) is generally thought to be negatively regulated by the PI3K pathways.

We have shown that a GSK3-inhibiting drug, CHIR99021, promotes the proliferative expansion of DN3a cultured with recombinant Delta Like4 and SDF1alpha. Here we show that developmental progression of either DN3a or DN3b is promoted by CHIR99021. Furthermore, inclusion of CHIR99021 allowed differentiation in the absence of preTCR- or Notch1-mediated signalling. Inactivation of GSK3 using CHIR99021 appears to antagonize IL-7-mediated inhibition of development at the DN stage. In addition to the effect on T cell development, CHIR99021 increased IL-7 dependent proliferation and caused enhanced cell recovery in these experiments. These experiments indicate a potentially important role for inactivation of GSK3 during the process of beta-selection. A stromal free culture system that promotes beta-selection may offer a new drug discovery platform for screening regulators of proliferation, differentiation and apoptosis.

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Lymphocyte interactions with hepatocytes: distinct mechanisms for T and B cells

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Hepatitis C virus (HCV) infects hepatocytes and causes chronic liver inflammation in the majority of infected individuals. Immune dysfunction is likely to contribute to both liver damage and a failure to resolve infection, yet little is known about the interactions of immune cells with hepatocytes. We investigated the role of the mechanisms involved in lymphocyte-hepatocyte interactions, with a focus on lymphocyte migration through the liver parenchyma and its consequences for immune cell effector function. Primary lymphocytes and hepatocytes were used in combination with hepatoma cell lines and replication competent HCV clones. *Ex vivo* lymphocyte migration assays were performed using biopsy material and tissue from explanted liver. Results were confirmed by *in vivo* observations using tissue sections from patients with end stage liver disease of viral and non-viral origin. Our experiments demonstrate the existence of novel interactions between T cells and hepatocytes that are regulated by HCV infection. We propose that the nature of T cell-hepatocyte interactions may have an impact on T-cell effector function and the outcome of anti-viral immune responses.

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Uptake of healthy, senescent and eryptotic red blood cells by macrophages

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Despite increasing recognition of the clinical importance of red blood cell (RBC) ageing and clearance, not least in the area of transfusion medicine, the mechanisms and consequences of their disposal by macrophages remain unclear. A major process associated with RBC ageing is a form of suicidal cell death that has been termed 'eryptosis', which has several similarities to apoptosis of nucleated cells. However, in contrast to the clearance of apoptotic cells, very little is known about the uptake of ageing and eryptotic RBC by phagocytic cells and its immunological consequences. The aims were to determine whether ageing of human RBC naturally *in vivo*, or during storage *in vitro*, or induction of eryptosis, altered their efficiency of uptake by macrophages. The effects of uptake on macrophage phenotype, as judged by cytokine production, were also compared with those induced by apoptotic neutrophils. We demonstrate that both aged RBC from fresh blood, fractionated on the basis of density, or stored RBC, are taken up by the macrophage cell line, J774 or human monocyte-derived macrophages with a significantly higher phagocytic index than younger cells. The highest efficiency of uptake was seen when eryptosis was induced by calcium ionophore treatment of RBC. When the effects on macrophage phenotype were compared, apoptotic neutrophil uptake reduced the production of inflammatory cytokines such as TNF-α, whilst eryptotic cells could have the opposite result. Such pro-inflammatory properties of effete RBC, if recapitulated *in vivo*, could contribute to pathologies reported to be associated with transfusion of blood after prolonged storage.

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Defining novel afferent signals in the lymphoid stress surveillance response

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It has recently been demonstrated that TCR gamma delta-expressing dendritic epidermal T cells (DETC) in murine epidermis can not only protect against cutaneous carcinogenesis, but can also initiate a systemic Th2-like immune response upon upregulation of the NKG2D ligand Rae1 on keratinocytes. The protective or pathophysiologic role of this functionality remains unknown. In order to investigate this more fully we have begun a study to find novel afferent stimuli that may initiate such a response upstream of the NKG2D pathway or other activatory signals. We are developing *in vitro* a coculture system of DETC and primary keratinocytes in order to use known DETC readouts as a bioassay for keratinocyte mediated immunomodulation in response to such stimuli. Stimuli under test include sensitizing agents known to lead to a type 2 immune response as well TLR/NLR ligands. In parallel with these murine studies we are examining the effect of the same stimuli on human keratinocytes in order to rapidly translate findings in mouse to the human situation. This study will increase our understanding of the role of cutaneous lymphoid stress surveillance in a wider context, potentially to include allergy and immunological responses to toxins and irritants. It has the potential to broaden the spectrum of agents known to initiate lymphoid stress surveillance as well as to illuminate underappreciated mechanisms by which particular agents may be affecting the immune system.

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Serine protease inhibitor-6 (Spi6) is required for dendritic cell priming of anti-viral CD8 T cell responses through protection from granzyme B

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Spi6 is a serpin inhibitor of granzyme B (GrB) and has anti-apoptotic function in cytotoxic T cells (CTLs), where leakage of GrB from cytotoxic granules into the cytoplasm triggers apoptosis. CD8α dendritic cells (DC) are professional antigen presenting cells responsible for cross-presentation of viral antigens in secondary lymphoid organs. Mature DCs have higher resistance to CTL-induced apoptosis *in vitro* compared to immature ones: when Spi6 is absent, this resistance is lost. However, the reproduction of these results *in vivo* has been unsuccessful and the role of Spi6 in protecting DC from CTL-mediated apoptosis is still under debate. Using mice deficient in Spi6 we focus on the role of Spi6 in DC survival during the priming of naïve and memory anti-Lymphocytic Choriomeningitis murine virus (LCMV) CD8 T cell responses. We show that upon maturation Spi6 is expressed by CD8αDC *in vivo*. In our model, Spi6 KO DC functionality was comparable to WT but their survival was impaired. This resulted in defective expansion of wild-type LCMV-specific CD8 T cells. A similar requirement for Spi6 was found for the DC priming of the expansion of memory CD8 T cells. GrB KO CD8 T cells rescued the priming defect in Spi6 KO mice both during primary and secondary responses, demonstrating GrB as the physiological target of Spi6 in DCs. This result identifies GrB as a major immunosuppressive agent controlling the DC priming of anti-viral T cell mediated immunity.

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Do exosomes from EBV-infected B cells protect from IgE-sensitization?

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Aim: To investigate the mechanisms behind how B cell derived exosomes in the context of EBV infection influence the outcome of IgE-mediated allergic reactions.
Background: EBV infection at 2 years of age among infants has been shown to have a protective effect on IgE sensitization (Nilsson *et al. J. Allergy Clin. Immunol.* 2005; Saghafian-Hedengren S. *et al*., *J. Allergy Clin. Immunol*. 2010). EBV infected B cells (LCLs) release exosomes, derived from multivesicular bodies (MVB) which harbor the viral latent membrane protein 1 (LMP1). LMP1 signaling can replace CD40 signaling in B cells *in vivo* and has unique features of inducing class-switch recombination (Rastelli *et al. Blood,* 2008). EBV also encodes a viral IL-10 homologue.
Results: Recently, we demonstrated that LCL-derived exosomes selectively target B cells (Vallhov *et al. J Immunol*. 2010). Currently, we are investigating the quality of exosomes secreted during primary EBV infection. Moreover, we are exploring the hypothesis that LMP1 harboring exosomes can induce class-switch recombination in bystander B cells, leading to reduced IgE sensitization.

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Stromal cell maturation in ectopic lymphoid organs is dependent on lymphocyte-derived signals

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Acquisition of lymphoid-like features is a hallmark of ectopic lymphoneogenesis. While the effects of local production of lymphoid chemokines/cytokines on leukocytes have been largely described, little is known about the signals regulating stromal cell activation in ectopic sites. We designed a novel inducible model of resolving ectopic lymphoneogenesis, in adenoviral infected murine salivary glands to evaluate the signals regulating leukocyte/stromal cell interaction and stromal cell activation in different phases of the inflammatory process.

Whole tissue disaggregation, qRT-PCR and histological analysis of the ectopic lymphoid structures in murine salivary glands cannulated with 10^8 p.f.u. of luciferase expressing adenovirus was performed at different time points post cannulation (p.c.) in WT and RAG mice. FACS analysis of collagenase digested stromal cells showed in WT mice stromal cell activation with significant increase in the percentage of fibroblastic reticular cells (CD45−GP38+ CD31− cells) already at day 8 p.c. associated with high ectopic lymphoid chemokine expression, peaking at day 15 p.c., when full acquisition of lymphoid features was observed in the aggregates. Digested cells from the KO mice showed similar peak followed, however, by a dramatic drop in the number of FRC in the later stages as compared to WT. Accordingly, a significant drop in lymphoid chemokine/cytokine expression was observed by qRT-PCR. Overall these data suggest that while innate immune system derived signals are able to induce acquisition of lymphoid feature by the stromal cells, maintenance of this lymphoid phenotype and full maturation with lymphoid chemokine/cytokine production is only acquired in presence of infiltrating lymphocyte derived signals.

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Differential effects on antigen presentation of anti-CD40 stimulation

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Antigen presenting cells (APC) such as dendritic cells, macrophages and B cells may be activated by stimulating the costimulatory molecule CD40, causing upregulation of costimulatory molecules and cytokine production. The aim of our study was to investigate the effect of activating APCs with anti-CD40 monoclonal antibody in the Non-obese diabetic (NOD) mouse model of autoimmune diabetes. *In vitro*, we stimulated splenocytes from the NOD mouse for 24 and 48 h using anti-IgM, anti-IgM + anti-CD40 and anti-CD40 alone. We examined activation and expression of costimulatory molecules and intracellular cytokines. We found that anti-CD40 stimulated upregulation of CD80 and CD86, but less than anti-IgM alone and the effect of anti-IgM + anti-CD40 was not additive. We observed greater upregulation of the cytokines IL-6 and IL-10 in CD19 positive cells at 24 h with anti-IgM + anti-CD40 stimulation compared with either stimulus alone. We then immunized G9TCR transgenic mice expressing the insulin-reactive CD8 T cell receptor from the highly diabetogenic T cell clone G9C8 with anti-CD40 alone or anti-CD40+ insulin peptide. We found increased intracellular cytokine production of IL-6 and IL-10 in CD19 positive cells, 3 and 6 days after intraperitoneal injection. When the mice were observed after immunization for diabetes, we found that anti-CD40 alone was not sufficient to cause diabetes but immunization with anti-CD40+ insulin peptide caused diabetes in the TCR transgenic mice. Further experiments are required to understand how activation of B cells in this context synergises with activation of other APC.

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Local, NKG2D-dependent, immune response of gd T cells to low-level UVB irradiation

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We have previously demonstrated that TCRgammadelta+ dendritic epidermal T cells (DETC) are protective against carcinoma formation in an epithelial chemical carcinogenesis model. However, the role of epithelial immune cells in the other common and physiologically relevant model of UVB-induced skin carcinogenesis is not well understood. When the exposed back skin of mice is subjected to low-level (up to 100 mJ/cm^2) UVB irradiation, within 6 h we observe DETC rounding, which is followed by a dramatic rearrangement of the DETC compartment, with loss of DETC from the epidermis while the DETC compartment in the hair follicles is maintained. Concomitantly we observe widespread nuclear accumulation of p53 in the basal keratinocytes, indicative of DNA damage repair response, and the induction of NKG2D ligand Rae1 in the epidermis. Since we have previously demonstrated that the NKG2D pathway is an important mechanism of DETC activation, we subjected NKG2D knockout mice to low-level UVB irradiation. In the absence of NKG2D, the loss of DETC from the epidermis is accelerated, as is the loss of DETC from the hair follicles. We also observe greater epidermal thickening and a significant increase in keratinocyte proliferation in the absence of NKG2D. We therefore propose that DETC may use the NKG2D receptor to recognize and limit the outgrowth of keratinocytes that have sustained DNA damage. These findings, as well as the role of DETC in the hair follicle, are subject to ongoing characterization.

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OX40/CD30 double KO animals are characterized by impaired immune response and defective ectopic lymphoneogenesis in a novel model of salivary gland inflammation

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OX40 and CD30 regulate memory T cell survival and activation. OX40/CD30 knockout mice provide a valuable tool to ascertain the relationship between memory, generation of autoimmunity and formation of chronic lymphocytic infiltrates. In this study we used a novel model of adenoviral induced tertiary lymphoid neogenesis in murine salivary glands in order to evaluate the effects that lack of OX40/CD30 plays on the local organization of the inflammatory infiltrate.

Significant reduction of the local immune response was observed in OX40/CD30 KO mice as compared to WT. In particular decreased number of T and B cells was observed by immunofluoresce (IF) in the tissue at different time points post infection with formation of smaller and less organized foci. FACS analysis confirmed a significant reduction in the CD4+ infiltrating cells ($P = 0.033$ day 8, $P = 0.0013$ at day 23 p.c.), in the memory, naïve and activated components. The number of fibroblastic reticular cells did not decrease. These data clearly demonstrate a role for OX40 and CD30 in the local aggregate organization and not only in the generation of the humoral response. These data were reflected in lower levels of anti-viral specific IgG response. Interstingly, while WT animals showed presence of ANA in 70% of sera, no ANA were detected in the knockouts. These data suggest an intimate link between generation of autoimmunity and memory and support a novel role for OX40/CD30 in the regulation of local lymphocytic aggregation, providing a therapeutic target for autoimmune diseases.

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The role of IKK subunits in lymph node organogenesis

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The importance of the lymphotoxin-beta receptor (LTbR) and downstream NFkB signalling in lymph node (LN) organogenesis has been clearly established as LNs do not develop in mice lacking these molecules. The role that the IKK signalling molecules play in this process has proved harder to define as complete genetic knockouts are lethal at the embryonic stage. Here we use WNT1Cre mice to dissect the contribution of these molecules to lymph node organogenesis. WNT1 is expressed by the mesenchymal cells of the head neck region including the CLN stromal cells, thus by placing floxed IKK genes under the control of WNT1 we can ablate their expression in CLNs but not other LNs. The IKK molecules are thought to be involved in signalling downstream of LTbR, therefore LN development should not be possible in the absence of these molecules. However, we show that CLNs are present with normal microarchitecture in mice where IKKa is under the control of WNT1. CLNs are also present in mice where IKKb is under the control of WNT1, however, although the microarchitecture of those nodes in normal, many of these nodes are grossly enlargened compared to those in wild-type controls.

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Pre-adipocytes give rise to lymph node stromal cells

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Lymph nodes (LN) are highly organised structures, distributed throughout the lymphatic vessel network, that provide an effective environment for antigen presentation to lymphocytes and generation of memory immune responses. Most LNs are embedded in the adipose tissue that develops in association with the lymphatic vasculature. LN anlage formation takes place during embryogenesis and, via haemopoietic crosstalk with innate lymphoid tissue inducer cells, involves the differentiation of mesenchymal cells into lymphoid tissue organiser cells. However, the developmental relationship between lymphoid tissue organiser cells and their surrounding adipocytes, and the molecular mechanisms that regulate the generation of these distinct lineages, remains elusive. Here we show that a common precursor cell, present in mouse embryos at the time of LN organogenesis, gives rise to both lymph node intrinsic organizer stromal cells and the adipocytes that reside within adjacent fat pads. Signaling through the Lymphotoxin-beta Receptor plays a key role in lineage choice of this progenitor population by inhibiting adipogenic differentiation and instead promoting lymphoid tissue stromal cell development. *In vivo* organogenesis experiments show that in the context of adult tissues, preadipocytes can differentiate into a variety of LN stromal cells that include organiser cells as well as capsular and LN medulla stroma. Thus, we show that adipose tissues contain precursor cells with potential for both adipogenic and lymphoid tissue stroma cells, which we suggest may also act as a source of other lymphoid structures associated with fat such as the milky spots of the omentum or fat associated lymphoid clusters.

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Outer membrane proteins of *Streptococcus suis*: immune evasion by interference with the complement pathway

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Streptococcus suis is a major swine pathogen and an emerging zoonotic agent, which has caused human infections in several parts of the world and also contributed to major outbreaks in China. It is a respiratory pathogen which can cause conditions including meningitis, pneumonia, arthritis and septicaemia in pigs as well as humans. The initial stages of host-pathogen interactions and also the mechanisms used by *S. suis* to subvert host immune responses are not clearly understood. A group of outer membrane proteins of *S. suis* belonging to Streptococcal histidine triad family have been predicted to be involved in complement evasion and invasion of host cells. My current results show that these proteins interfere with both classical and alternative pathway of complement activation by interacting with complement components, mainly C3. Experiments are also under progress to study their ability to interact with respiratory epithelial cells and activate Toll-like receptors resulting in cytokines production.

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The PI3-kinase/Akt signalling pathway influences integrin adhesion in lymphocytes

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In the immune system, integrin-mediated adhesion is important for lymphocyte recirculation, activation and effector functions. The PI3-kinase pathway has been previously implicated in the regulation of lymphocyte integrins. We have now investigated the roles of PI3-kinase and two downstream kinases, PDK1 and Akt, in integrin-mediated functions in lymphocytes. The pre-treatment of murine naive B cells and effector T cells with PI3-kinase inhibitors resulted in a significant reduction in adhesion to ICAM-1, suggesting a role for PI3-kinase in regulating aLb2 (LFA-1) integrin-mediated adhesion. Similarly, PDK1-deficient lymphocytes also showed a decrease in binding to ICAM-1. Furthermore, the inhibition of Akt in B cells reduced binding to ICAM-1 in a static adhesion assay, as well as under shear-flow conditions. In contrast, in effector T cells, adhesion to ICAM-1 in static conditions, but not under shear-flow, was significantly reduced in Akt inhibitor-treated cells. Such Akt inhibitor-treated effector T cells required significantly lower forces for detachment from the ICAM-1 ligand when compared to untreated T cells, as measured using Atomic Force Microscopy, confirming the requirement for Akt in LFA-1-mediated adhesion. Among the possible downstream effectors of Akt is the small GTPase, Rap1. Levels of Rap1-GTP were decreased in mouse B cell line, A20, following treatment with an Akt inhibitor. We propose that the PI3-kinase/Akt pathway may be an important signalling axis in the regulation of lymphocyte integrins, but that it plays different roles in naive B cells and effector T cells.

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Combinatorial effects of ambient air pollution particulate matter and allergen on respiratory cells

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Ambient air pollution is proposed to play a role in the exacerbation of asthma, however the mechanisms are unclear. The airway epithelium is the first barrier of defence against foreign particles, and is thought to modulate the function of underlying dendritic cells. We propose that pollution particulate matter (PM) induces oxidative stress in human bronchial epithelial cells (HBEC), leading to the generation of inflammatory and pro-allergic cytokine by HBEC. Recent independent studies show allergens can directly activate airway HBEC. We propose that exposure to allergen and PM will result in greater levels of HBEC activation than with either agent alone.

Results: The human bronchial epithelial cell line, 16HBE14o-, was cultured with PM (NIST – National Institute of Standards and Technology SRM2397) and cytokine secretion at 24 h assessed by Cytokine Bead Array (CBA). NIST induced production of IL-6, GM-CSF and IL-8. Comparable data were obtained using PM samples from monitored sites in the London Low Emission Zone. The level of cytokines produced correlated with oxidative potentials of these samples ($r = 0.8$). Conversely, sulforaphane, an inducer of anti-oxidative enzymes, inhibited cytokine production in response to PM. IL-8, GM-CSF and IL-6 levels were not significantly increased by cat allergen alone, but combining allergen with NIST significantly increased cytokine production above NIST alone. This correlated with a greater level of oxidative stress as assessed by the dichlorofluorescein assay ($P < 0.05$).

Conclusion: Cat allergen and PM act together on HBEC to enhance their inflammatory phenotype. A role for oxidative potential of PM is implicated.

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Impact of resistin like molecule beta on clinical status of human colorectal cancer patients

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Previous studies have indicated that resistin like molecule beta (RELM β), an intestinal goblet cell specific protein, is markedly enhanced in human colorectal cancers. We hypothesized that there is an association between the expressions of RELM β with CD133 a cancer stem cell marker and CD3 a tumor infiltrating lymphocytes (TIL) in colorectal cancers. The aim of this study was to examine the association between RELM β expressions with the expression of CD133, CD3 counts and to correlate this expression with various clinicopathological parameters in colorectal cancer using immunohistochemistry. Of the 120 colon cancer patients studied 120 (100%) tested positive for expressions of RELM β, CD133 and CD3 counts contrasting sharply with normal colon mucosa membrane which was negative or weakly positive. This positivity in colorectal cancer was correlated significantly between histological grade with RELM β ($\chi^2 = 42.550$, $P \leq 0.001$), CD133 ($\chi^2 = 7.372$, $P = 0.007$) and CD3 ($Z = -8.003$, $P \leq 0.001$). Dukes' stages shows significant association against RELM β ($\chi^2 = 51.0$, $P \leq 0.001$), CD133 ($\chi^2 = 7.988$, $P = 0.0046$) and CD3 ($P < 0.001$). RELM β shows no significant association with age, tumor location and gender. These findings support the evidence of association between RELM β, CD133 and CD3 in colorectal cancers and suggest that further investigation is warranted to explore its possible role as a prognostic or diagnostic marker.

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Role of miR-22 in regulation of cell cycle specific gene expression in early phase of Epstein–Barr virus infected B cells

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MicroRNAs are small non-coding RNAs that negatively regulate gene expression. It has been reported that miRNAs are expressed in mammalian and plant cells. The recent study showed that virus also encodes miRNAs. Epstein–Barr virus (EBV) is a dsDNA human gamma herpes virus which infects more than 90% of human populations. It is associated with the development of malignancies such as Burkitt's lymphoma, nasopharyngeal carcinoma, and EBV-associated gastric carcinoma. EBV has recently been shown to encode miRNAs. Although we can predict that the small size of miRNA precursors makes them potentially ideal for use by viruses as inhibitors of host cell defense pathways, the function of most of them and expression patterns are still unknown.

In this study, we showed that one of EBV viral miRNA, miBHRf1-3, was expressed during an initial infection step and can inhibit $p21^{Waf1}$ translation by binding on 3′ UTR of $p21^{Waf1}$. We also demonstrated that the expression of level of miR-22 is also increased simultaneously. MiR-22 is known as a tumor suppressor, thus, it is related to cell division, proliferation, development and cell cycle. Here we show that miR-22 could also suppress $p21^{Waf1}$ by binding on 3′ UTR of $p21^{Waf1}$. To determine the function of viral miRNAs, we analyzed cell cycle progression and apoptosis in EBV positive cells with or without transfecting 2′-O-Methyl oligonucleotides, miRNA inhibitor. 2′-O-Me-mir-BHRF1-3 resulted in an increase of apoptosis in EBV infected cells, implicating that mir-BHRF1-3 and mir-22 function in cell survival maintenance.

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A novel model to investigate T cell – oligodendrocyte precursor cell interactions

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Although the immune system is known to be involved in Multiple Sclerosis pathogenesis, it is also implicated in remyelination. *In vivo* studies have shown T cells to be necessary for efficient remyelination, however the mechanisms by which this occurs are poorly understood. We hypothesise that interactions between CD4+ T cell subsets and oligodendrocyte precursor cells (OPCs) may drive OPC maturation and potentially alter T cell phenotypes and influence remyelination. In order to investigate this, we sought to develop an *in vitro* model whereby the reciprocal effect of T cells on OPCs could be further studied. OPCs were isolated from neonatal murine forebrains and digestion, separation and culture conditions were optimised. Using FACS or magnetic beads, OPCs were selected for either NG2 or O4, early and late OPC markers respectively. OPCs were cultured in conditions which supported proliferation, before being co-cultured with CD4+ T cell subsets which were polarised to distinct subsets. As OPCs cannot be grown in serum, we tested a range of co-culture conditions. By combining the OPC serum-free media and serum-free RPMI, we found that both cell types were supported and the supplements contained in the OPC media were sufficient for T cells to thrive in the absence of serum. The maturation was quantified by immunofluorescence using a marker of mature oligodendrocytes (myelin basic protein). T cell phenotype was characterised by flow cytometry. This study has generated a novel T cell – OPC co-culture model which lends itself to investigation of OPC maturation, a key process in remyelination.

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Mesenchymal stem cells co-cultivation with mononuclear cells treated with phytohemagglutinin interferes with lymphocyte late apoptosis/necrosis

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Mesenchymal stem cells (MSC) represent an adult population of non hematopoietic stem cells, which can differentiate into a variety of cell types and are able to modulate immune responses.

The objective of our study was to investigate alterations in T cell apoptosis by co-cultivation with MSC. Mesenchymal stem cells were obtained from human bone marrow discarded cells (Ethical Committee Approval – Process 10/1412); MSC were characterized by flow cytometry immunophenotyping and by the multilineage differentiation. Peripheral blood was obtained from healthy volunteers after signed informed consent; and processed by gradient density to obtain peripheral blood mononuclear cells (PBMC).

PBMC were stimulated with 1 μg/ml phytohemagglutinin and co-cultivated for 24 and 48 h with MSC, and PBMC cultivated without MSC for 24 and 48 h served as control.

Briefly; non adherent cells were stained with Annexin V (FITC), Propidium Iodide (PI) and CD3 (APC). We observed that phytohemagglutinin stimulated T cells (CD3) co-cultivated with MSC presented less late apoptosis/necrosis than the cells cultivated without MSC. After 24 h the mean percentage of late apoptosis/necrosis without MSC ($n = 8$) was (24 ± 9) and with MSC co-cultivation ($n = 8$) was (19 ± 10). After 48 h the mean late apoptosis/necrosis without MSC ($n = 8$) was (41 ± 11) and the with MSC co-cultivation ($n = 8$) was (28 ± 13), $P < 0.05$.

Than we further investigated the cells stimulated by 24 h with phytohemagglutinin, than washed and co-cultivated with MSC by 24 h and we observed a direct or indirect action of phytohemagglutinin in the MSC that have caused at least part of this effect.

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CD1d splice variants: immunoregulatory role in liver metastasis

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iNKT cells, powerful anti tumour cells, have been found to be functionally compromised in metastatic liver. These cells are restricted by CD1d, a molecule which presents glycolipids to iNKT cells resulting in their activation. Evidence suggests CD1d is spliced into multiple isoforms. Bioinformatic analysis of ESTs confirmed this and detected the presence of a putative soluble splice variant of CD1d which may act as a regulator of iNKT activity. Primer sets were designed to detect the complete CD1d transcript and the soluble splice variant which we called SP2. Using these, qPCR revealed higher levels (>10-fold) of SP2 in metastatic liver when compared to healthy tissue. We aim to examine the effects that these splice variants have on hepatic iNKT cells using liver perfusate as a source of healthy hepatic iNKT cells.

In human liver Vα24Jα18$^+$ iNKT cells, while significantly expanded when compared with other sites, are less abundant (approximately 4−5% of hepatic T cells) and therefore difficult to study. Here we describe a relatively small but accessible population of liver iNKT cells. *Ex vivo* perfusion of donor livers releases a significant population of hepatic mononuclear cells (HMNCs). From 18 transplants, yields of $15-228 \times 10^6$ HMNCs were obtained. Almost 25% (13−37%) of these were CD3+ CD56− T cells, 40% (24−53%) were CD3−CD56+ NK cells, 5% (3−7%) were CD3+CD56+NKT cells, 0.5% were 6β11+ iNKT cells and 0.18% were Vα24+ Vβ11+ cells. We aim to expand this population of iNKT cells in order to test the function of CD1d isoforms.

Immunological Basis of Disease

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Anti-cardiolipin autoantibody expression as a potential biomarker for deep vein thrombosis with anti-phospholipid syndrome

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Background: This study sought to determine the frequency rates of anti-cardiolipin (aCL) dependent on the presence of β_2-GPI, anti-β_2-glycoprotein I (aβ_2-GPI), and anti-phosphatidyl serine (aPS) IgG autoantibodies among patients with deep venous thrombosis of legs.
Methods: Fifty patients with deep vein thrombosis of legs and 50 control subjects recruited from Kurdistan Region, Iraq were evaluated. All cases were under 50 years-of-age and had no recognizable risk factors. Using ELISA to detect the presence of IgG isotype of aCL, aβ_2-GPI, and aPS autoantibodies in their blood.
Results: The frequency of aCL was 15/50 (30%), aβ_2-GPI was 13/50 (26%), and aPS was 5/50 (10%) among patients. In contrast, only aCL was detected in 2/50 (4%) of control subjects. Of all the aCL^+ cases, the incidence of patients having the combined profile of aCL + aβ_2-GPI was 13/15 (86.7%) and of aCL + aPS was 5/15 (33.3%). Only 8/15 (53.3%) of these aCL^+ patients also expressed aβ2-GPI^+ in the absence of aPS. The frequency of patients expressing all three markers was only 5/15 (33.3%). In none of the APS positive patients were aβ_2-GPI or aPS expressed in the absence of aCL. Conversely, IgG aCL as a sole marker was seen in 2/15 (13.3%) of these patients (i.e. in absence of either other markers).
Conclusions: It can be concluded from these studies that among the three major forms of APLA examined, the presence of IgG aCL autoantibodies appeared to correlate best with patients having DVT of legs who were concurrently suffering APS.

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Smoking and regulation in chronic obstructive pulmonary disease (COPD)

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Introduction: T regulatory cells are a subset of $CD4^+$ T helper cells. These cells are important for maintaining self tolerance and the production of anti inflammatory cytokines in chronic inflammation, such as COPD and other diseases of the lung. Increased levels of $CD4^+CD25^+$ have been observed in COPD patients with acute exacerbations and emphysema.
Method: Twenty seven participants were recruited, 11 with COPD, nine healthy smokers without COPD and seven healthy non smokers. A sample of venous blood was taken and mixed with flow count beads. The samples were analysed on the FC500 flow cytometer straight away. The absolute number of $CD3^+CD4^+CD25^{hi+}$ was calculated as cells/μl for every participant.
Results: In peripheral blood the absolute numbers of $CD3^+CD4^+CD25^{hi+}$ cells are significantly increased in healthy smokers ($P = 0.033$) and patients with COPD ($P = 0.018$) compared to non smoking healthy controls. Interestingly ex-smokers within the COPD group are observed as having lower numbers of $CD3^+CD4^+CD25^{hi+-}$ compared to those who still smoke.
Conclusion: Due to similar numbers of $CD3^+CD4^+CD25^{hi+}$ in the smoking and COPD group this indicates the results are due to the act of smoking it self not the disease alone. The smoke particles being inhaled by both groups are activating the T regulatory cells in the periphery. There seems to be lower levels of inflammation in the smokers with COPD that have given up in the last few years.

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Evolutionary history of the low-affinity Fc gamma receptor copy number variable locus: diversity, disease and helminth infection

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Low-affinity Fc receptors for immunoglobulin G (IgG) are expressed on a variety of leucocytes and play an important role in immune responses to pathogens. Copy number and functional single nucleotide variation in the low affinity FCGR region is associated with lupus, malaria, and possibly rheumatoid arthritis. We analysed variation at this locus in a global survey of 946 individuals from 51 populations. We found no large differences in copy number distribution together with little association with flanking SNP haplotypes suggesting a high recurrent mutation rate of this CNV. Coalescent analysis of population data suggests a mutation rate of about 0.1% per generation. A model of recurrent duplication and deletion mediated by non-allelic homologous recombination is supported by breakpoint mapping of homozygous deletions. Given the functional relevance of the sequence variation typed, infectious disease burden may be involved in shaping variation. Indeed, helminth pathogen richness is significantly correlated with the frequency of the NA1 variant of FCGR3B ($P = 0.0018$) and an active form of the FCGR2C receptor ($P = 0.0005$). Maximum-likelihood analysis of sequence evolution in mammals supports a model where positive selection acted on lineages with high levels of helminth infection ($P = 0.006$). Positive selection has acted on a subset of amino acids in FCGR3 which were mapped to the crystal structure and formed three patches on the receptor likely to influence the interaction with IgG.

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The formation of fused monocyte giant cells in common variable immunodeficiency

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The fusion of peripheral monocytes to form multinucleate giant cells may be related to granuloma formation in inflammatory diseases. Giant cells of common variable immunodeficiency (CVID) and normal individuals were examined under electron and confocal microscopes after culture in a range of cytokines, supernatants and mitogens. CVID peripheral blood mononuclear cells had a great range but on average fused more quickly and with higher numbers of nuclei in a twofold greater tendency to form giant cells in culture medium without cytokines than normal. Addition of IL4, GMCSF, IFNg, TNFa and T cell conditioned media further induced normal and particularly CVID giant cell formation and combinations of cytokines and monokines acted synergistically in promoting monocyte fusion. Treatment with anti INFg antibody reduced normal giant cell formation particularly, indicating a greater predisposition of peripheral CVID cells to fuse, while a greater tendency of CVID cells to fuse with immunoglobulin conditioned media my indicate the contribution of IVIG treatment in granuloma formation. CVID and normal giant cells expressed similar levels of MHC class II and costimulatory molecules and FC receptors and demonstrated metabolic and phogocytic activity with bacteria, yeast and fluorescent carboxilated beads. A 2–5 fold greater tendency to form giant cells was induced in peripheral CVID monocytes by an extensive range of monokines, inflammatory lymphokines and T cell supernatants. CVID and normal cell giant cells were metabolically active and phenotypically similar.

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The role of alveolar macrophages in the resolution of house dust mite induced allergic airways disease

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Allergic asthma is a chronic inflammatory disease of the lung. Deficiencies in pro-resolving mechanisms may contribute to the persistence of inflammation in the lung. Alveolar macrophages are considered to have a critical regulatory role in the lung, but their interactions in the allergic lung are not well understood. This study was to investigate the role of alveolar macrophages in the allergic lung during resolution of HDM induced allergic airways disease. Disease parameters were measured at 4 h, 7 and 13 days following cessation of allergen exposure. Airway hyper-reactivity was sustained 7 days post challenge compared to PBS treated controls, returning to baseline by 13 days, accompanied with concomitant levels of Th2 lymphocytes and eosinophils. Alveolar macrophage numbers increased at 4 h and remained significantly elevated at 7 and 13 days (5.96×10^5 and 4.60×10^5 versus 3.03×10^5 cells/ml, $P < 0.05$). Depletion of alveolar macrophages during the resolution phase was carried out using i.t administration of clodronate encapsulated liposome. This resulted in a significant increase of Th2 lymphocytes at 13 days compared to mice receiving liposome control (26.9×10^3 versus 13.0×10^3 cells/ml, $P < 0.05$). The adoptive transfer of naive alveolar macrophages during resolution did not alter airway resistance; however, total cell numbers (89.8×10^5 versus 121.9×10^5 cells/ml, $P < 0.05$) and eosinophils (7.95×10^5 versus 17.9×10^5 cells/ml, $P < 0.05$) were significantly decreased. These data indicate that distinct pathways are responsible for resolution of allergic inflammation and alveolar macrophages have a specific role to play.

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Decreased CD4+ and CD8+ effector memory T lymphocyte populations in thyroid-associated ophthalmopathy

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Purpose: Relative proportions of peripheral blood memory T cell subsets vary in a number of inflammatory and autoimmune conditions. The aim of this study was to definitively characterise $CD4^+$ and $CD8^+$ T cell memory populations in Thyroid-Associated Ophthalmopathy (TAO), an inflammatory disease of the orbit associated with Graves' disease.

Methods: Multicolour flow cytometry was used to analyse CD4, CD8, CD45RO and CCR7 expression, and IFN-γ and IL-17 production, by PMA/ionomycin-stimulated PBMCs from 20 subjects with TAO and 11 age- and sex-matched healthy controls. Naïve and effector memory populations were defined as $CD45RO^-$ $CCR7^+$ and $CD45RO^+$ $CCR7^-$, respectively. Statistical analysis was undertaken with Mann–Whitney *U*-test.

Results: There were significant increases in the proportion of naïve T cells in both CD4+ (median 71% versus 40%) and CD8+ (median 74%% versus 46%) compartments, and similar reductions in effector memory CD4+ (median 10% versus 16%) and CD8+ (median 9.7% versus 23%) cells in TAO subjects, compared with controls. Consequently, there was lower production of IFN-γ (median 4.4% versus 12% for CD4+ and median 6% versus 16%, for CD8+) and IL-17 (median 0.45% versus 1.3%, for CD4+ and median 0.38% versus 0.59%).

Conclusions: This study demonstrates reduced cytokine-producing effector memory CD4+ and CD8+ T cells in TAO. Skewing of memory T cell populations may represent dysregulated lymphocyte homeostasis, with preferential generation or survival of naïve T cells, or increased loss or sequestration of effector memory T cells in inflamed tissues. Future experiments will aim to clarify the immunopathogenic significance of this observation.

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T cell-derived IL-6 and IL-13 drive dermal fibrosis: implications for systemic sclerosis

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Systemic Sclerosis (SS) an autoimmune disease of unknown aetiology that is characterised by inflammation. Immune abnormalities in the disease include T cell activation and a host of proinflammatory cytokines. Tumour necrosis-α (TNF-α) is a pro-inflammatory cytokine that may be involved in disease pathogenesis and is upregulated in SS. TNF-α signals through two receptors. The aim was to investigate the role of TNF-α in T cells and the role of pro-inflammatory cytokines in SS. T cells from SS and controls were analysed for TNF-α receptor using flow. Specific mutant ligands that are recombinant for TNF-α receptor subtypes or soluble TNF was used to examine downstream effects. T cell-derived cytokines were measured using ELISA and subsequent cytokines neutralised with antibodies or isotype controls and collagen I measured. T cells were present in high numbers in the skin of patients. TNF-αR II was elevated in T cells from both the skin of affected patients and also T cells from blood compared to healthy controls. Mutant ligands to receptor subtypes leads to elevated Interleukin-6 and also IL-13 expression from healthy and SSc donors. SS donors have a much higher constitutive level of both cytokines without the addition of TNF-α ligands. Medium also upregulated Collagen I expression by 20-fold after incubation with TNF. Suppression of T cell derived cytokines IL-6 and IL-13 in combination by neutralising antibodies leads to an attenuated increased collagen I mRNA expression. T cells are activated '*in vivo*' and secrete the cytokines IL-6 and IL-13. IL-6 and IL-13 work synergistically.

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Molecular mimicries of linear epitopes between pancreatic glutamic acid decarboxylase (GAD65) and proteins from *Escherichia coli*

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Type1 diabetes (T1D) is characterised by autoimmunity to β-cell auto-antigens such as GAD65 and insulin. GAD auto-antibodies target epitopes whose isoforms may present in microorganisms, and contribute as an environmental factor in development of diabetic autoimmune responses. This study searched a range of microorganisms for GAD 65 mimicries. Purified GAD protein was produced using Halo-Tag technology in Chinese Hamster Ovary (CHO) cells, and used to stimulate production of GAD antiserum in mice. The GAD antiserum was titrated and used to screen total protein samples from 40 bacteria and five yeasts, using a dot blot technique. Interestingly, strong immunological reaction was seen with bacteria of the Enterobacteriaceae family. The positive samples were subjected to western blotting which produced immune active protein bands. For *E. coli* four bands were detected (40, 36, 22, 18 kDa). These were identified using mass spectrometry as an outer membrane protein A, formate dehydrogenase, superoxide dismutase and DNA starvation protein, respectively. These proteins expressed significant epitopes 1, 4, 4, 2, respectively, with strong homology (up to 70%) with the GAD epitopes. It was found that one epitope from *E. coli* was highly similar to several epitopes on GAD, whereas other epitopes from *E. coli* were similar to a single and different epitope on GAD. All these epitopes occur at the C-terminal region of GAD (residues 419–565), a region previously reported to be targeted by auto-antibodies. This suggests that those epitopes may be inducers for autoimmunity particularly in individuals who are immune-compromised or genetically predisposed for T1D.

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Characterization of the rabbit CD200R family and investigation of a possible interaction between rabbit CD200R and M141 (a viral CD200 homolog)

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CD200R is an inhibitory receptor expressed on various leukocytes. It is responsible for down-regulating immune responses upon engagement with its ligand CD200, which is expressed on a wide range of cells. The immune suppression generated by this interaction has been shown to be targeted by several DNA viruses. These pathogens possess viral CD200 homologs (vCD200) which they use to bind with host's CD200R in order to escape from immune response. However in contrast to well characterized vCD200 molecules seen in herpes viruses, the function of vCD200 molecules in pox viruses is not known. In this study we have investigated the interaction between M141 (vCD200 in rabbit myxoma viruses) and rabbit CD200R. For this purpose full length rabbit CD200 and CD200R sequences were identified by aligning genomic sequence of *Oryctolagus cuniculus* with sequence of CD200 and CD200R from known species. One inhibitory and two possible activating members of CD200R family were characterized on chromosome 14. Soluble purified recombinant rabbit CD200R was shown to bind immobilized rabbit CD200 (K_D = 3.27 μM) using surface plasmon resonance but there was no binding with M141. Stable cell lines expressing full length M141 and rabbit CD200 were generated and they were incubated with fluorescent beads coated with rabbit CD200R. Beads were shown to bind to rabbit CD200 expressing cells but not to M141 expressing cells. These results suggest a CD200R independent role for M141, the viral CD200 homolog.

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β-Defensin genomic copy number is associated with HIV viral load and immune reconstitution in sub-Saharan Africans

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AIDS, caused by the retrovirus HIV, is the leading cause of death of economically-active people (age 15–59) in sub-Saharan Africa. It is characterised by high HIV viral load and reduced (<200 cells/mm^3) CD4$^+$ T-cell count. β-defensins are broad-spectrum antimicrobial genes that are also chemotactic for dendritic cells and T-cells through the CCR6. β-defensin genes have previously been shown to be copy number variable, that is, different individuals have different numbers of the same gene. In this cohort study we analysed the relationship between β-defensin genomic copy number and HIV viral load immediately prior to initiation of retroviral treatment in 627 Ethiopian and 325 Tanzanian HIV patients, some co-infected with tuberculosis. We also measured the response to Highly Active Antiretroviral Therapy (HAART) by measuring follow-up CD4+ T-cell counts and viral load counts in a subsection of these patients. We found that high β-defensin copy number was associated with increased baseline HIV viral load, independent of co-infection with tuberculosis and population of origin. We also found that high β-defensin copy number was associated with impaired immune reconstitution after initiation of HAART, as measured by CD4 count up to 48 weeks follow-up and virological failure (persistence of viremia with viral load >200 copies/ml). Given the known chemotactic role of β-defensins, our data suggest a model where β-defensins recruit HIV-permissive Th17 lymphocytes to mucosal sites via the chemokine receptor CCR6.

E. Hollox and E. Aklillu are joint senior authors.

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Characterising the effects of treating dendritic cells with the small intestinal extra-cellular matrix proteins, tissue transglutaminase (TG-2) and fibronectin

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Introduction: Dendritic cells (DCs) are part of the innate immune system with a key role in modulating T cell mediated immune responses. Coeliac disease is caused by inappropriate activation of such a response leading to small intestinal inflammation when gluten is ingested. Tissue transglutaminase (TG-2) is an extracellular matrix (ECM) protein and has an established role in coeliac disease; however, no work to date has examined its impact on DCs. The aim of this study was to investigate the effect of the small intestinal ECM proteins, tissue transglutaminase (TG-2) and fibronectin (FN), on human DCs by including these proteins in DC cultures.

Methods: The study used flow cytometry to determine the effect of TG-2 and FN on DC phenotype and endocytosis, and scanning electron microscopy to assess morphological changes. Pre-treated DCs were cultured with naïve, autologous, T cells and subsequent T cell proliferation and cytokine profile determined. Furthermore, a co-culture of DCs, intestinal epithelial cells and TG-2 was established.

Results and discussion: The data indicate that TG-2 affected DCs in a concentration-dependent manner. High concentrations of TG-2 were associated with a more mature phenotype and increased ability to stimulate T cells. Lower concentrations led to maintenance of an immature phenotype, with persistence of endocytic ability and absence of T cell stimulation. Data on the effect of fibronectin were less conclusive. These data provide support for an additional role for TG-2 in coeliac disease and demonstrate the potential of *in vitro* modelling of coeliac disease pathogenesis.

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Clocks determine outcome of parasitic worm infection in mice

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An appropriate immune response is essential for survival in the context of infection. Many important cells and molecules in the immune response fluctuate following a daily rhythm, influencing the ability to respond to acute infection.

C57 BL6 mice were infected with a high dose (200 eggs) of *Trichuris muris*, a nematode parasite dwelling in the large intestine, at 7 AM or 7 PM. The progress of infection was monitored by worm counts, serum antibody levels and cytokine production in the mesenteric lymph nodes in mice culled on Day 13, Day 21, Day 25 and Day 28. Mice infected at 7 AM expelled the worm burden significantly earlier than those infected at 7 PM. 7 AM mice exhibited a stronger Th-2 type response, required for worm expulsion, compared to 7 PM mice.

This experiment shows that the speed of resolution of an acute infection over 3–4 weeks depends on the time of day of initial infection.

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The retinoic acid receptor agonist Am80 increases mucosal inflammation during an intestinal helminth infection of mice

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Vitamin A metabolites, such as *all-trans*-retinoic acid (RA) act through the nuclear receptor retinoic acid receptor (RAR) to regulate gene transcription. The role of RA in the regulation of chronic inflammation is largely unexplored, despite the fact that vitamin A is used to reduce morbidity during chronic-infection of helminth parasites in children and adults. Here, we use *Trichuris muris* infection of mice as a biologically-relevant model of chronic mucosal inflammation and treat with an RARα/β agonist (Am80). Critically, we show, for the first time, that rather than playing an anti-inflammatory role, Am80 actually exacerbates helminth-driven inflammation, described by an exaggerated crypt hyperplasia and an increased cellular infiltrate, and that these pro-inflammatory effects are IL-6 dependent. This study therefore presents novel data showing a pro-inflammatory role of retinoic acid in *T. muris* infection, and gives an important insight into the function of RAR in immune responses of the gut mucosa.

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Multi-parameter approaches to monitor the pathogenesis of collagen antibody induced arthritis (CAIA) in mast cell deficient mice

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Distinct immune cell populations are implicated in the development of rheumatoid arthritis (RA), however the involvement of mast cells (MC) has received much less attention. MCs are resident in the inflamed joint during RA and could trigger joint destruction directly by interaction with dendritic cells or T cells, or indirectly by release of a multitude of cytokines, proteases and other immunological mediators. In order to determine the role of mast cells in the subtle and early changes in joint pathology in inflammatory arthritis, we have employed *in vivo* imaging of multiple fluorescent and bioluminescent disease markers.

MC deficient Kit-^{Wsh} and their C57BL/6 wild type controls were injected i.p. with anti-collagen type II antibodies and arthritis synchronised with an LPS boost i.p. on day 3. Mice were injected with fluorescent probes activated by MMPs and cathepsins on day 6 post antibody injection. Mice were injected with bioluminescent probes for myeloperoxidase daily. Fluorescent and bioluminescent signals were quantified from the planar images. There was a positive correlation between arthritic score and cathepsin activation, as well as between myeloperoxidase activity and neutrophil influx. This study begins to give us insight into the molecular disease processes in CAIA.

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CD25 expression defines novel subsets of naïve human CD4+ T cells

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Previous studies from our group (Nat Gen 2009) have identified a MS and T1D autoimmune associated polymorphism within the IL-2RA gene region regulating CD25 expression by naïve CD4$^+$ T cells. CD25^{pos} naïve CD4$^+$ T cells respond to lower concentrations of IL-2 than their CD25^{neg} counterparts. Analysis of more than 200 healthy donors from genotype-selectable human bioresource demonstrated that the expression of CD25 on naïve CD4+ T cells increases with age regardless of sex and is increased in females. The protective *IL2RA* genotype prevents the age related increase of CD25 on naïve CD4+ T cells.

In order to characterise further CD25^{pos} naïve CD4+ T cells we assessed the expression of molecules that differentiate naïve and memory T cells on the surface of CD25^{pos} versus CD25^{neg} naïve CD4+ T cells: CD27, CD28, CD45RO, CD62L, CD69, CD183, CD194, CD195, CD196, CD197 and HLA-DR. Expression of these molecules did not discriminate CD25^{pos} naïve CD4+ T cells from their CD25^{neg} counterparts. Since a phenotypic difference was not observed, gene expression microarray analysis was performed and the results confirmed the naïve status of the CD25^{pos} subset.

Parallel analysis of CD25 and CD31 (a marker of thymic naïve CD4+ T cells that include recent thymic immigrants) expression demonstrated that CD25 is expressed on CD31^{pos} naïve CD4+ T cells; however, the frequency of CD25^{pos} cells is increased twofold amongst CD31^{neg} central naïve CD4 T cells. Ongoing studies are focused on the functional consequences of CD25 expression on the CD31^{pos} and CD31^{neg} naïve CD4+ T cell subsets.

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B-Cell growth factors in autoimmune blistering skin disease

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Autoimmune blistering diseases represent a heterogeneous group of disorders characterized by pathogenic circulating autoantibodies to structural components of skin and mucosal membranes. BAFF (B cell activating factor, TNFSF13B) and APRIL (A PRoliferation Inducing Ligand, TNFSF13A) both belong to the tumour necrosis factor superfamily. They are crucial mediators of B cell survival and function and over-expression of these ligands has been shown to promote autoimmunity. Elevated serum levels of BAFF and/or APRIL have been reported in several autoimmune diseases and may correlate with disease activity and/or titres of pathogenic autoantibodies.

We were interested in evaluating the role of BAFF and APRIL in the pathogenesis of autoimmune blistering skin disease. Circulating APRIL levels were elevated in patients with the sub-epidermal blistering conditions bullous pemphigoid (n = 50; mean = 25.8 ng/ml, range 6.4−286.9 ng/ml) and epidermolysis bullosa (EBA) (n = 19; mean = 18.3 ng/ml, range 1.3−69.4 ng/ml), but not in patients with intra-epidermal blistering disorders pemphigus vulgaris (n = 25; mean = 9.2 ng/ml, range 5.8−17.9 ng/ml) or pemphigus foliaceus (n = 13; mean = 11.3 ng/ml, range 2.4−20.2 ng/ml). Interestingly, BAFF levels were not elevated in any disease state but increased significantly following B-cell depletion therapy with rituximab in association with B cell recovery. We were unable to observe any relationship with antibody levels or disease activity.

Our data suggest that different B-cell control mechanisms exist in sub-epidermal immunobullous disease (pemphigoid and EBA) compared with pemphigus variants, where blistering is intra-epidermal. Consequently, therapeutic interference with BAFF and APRIL pathways may have differential effects in these disorders.

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Regulatory T cells exhibit reduced phenotypic stability upon pro-inflammatory challenge in autoimmune hepatitis

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Background: Expression of the ectoenzyme CD39 contributes to Treg suppressive ability by initiating an ATP hydrolysis cascade culminating in the production of the immunosuppressive molecule adenosine. Recently, expression of CD39 has been associated with Treg phenotypic stability under pro-inflammatory conditions. In autoimmune hepatitis (AIH) $CD4^{pos}CD25^{high}$ regulatory T cells (Tregs) are numerically and functionally defective, failing to suppress T cell-mediated immune responses targeted to the liver.
Aim: To investigate the frequency and phenotypic stability of $CD39^{pos}$ Tregs in AIH and in health.
Patients and methods: The phenotype and cytokine profile of circulating Tregs from 24 AIH patients and 24 healthy subjects (HS) was assessed by flow cytometry. Analysis was performed at baseline and after exposure to anti-CD3/CD28 T cell expander or the pro-inflammatory cytokines IL1β and IL6.
Results: At baseline, $CD39^{pos}$ Tregs were less numerous in AIH patients than HS and displayed a trend towards higher CD127 expression and reduced FOXP3 mean fluorescence intensity. Exposure to T cell expander increased the frequency of $IFN\gamma^{pos}CD39^{pos}$ Tregs in AIH but not in HS. Although the frequency of $IFN\gamma^{pos}CD39^{pos}$ Tregs augmented after treatment with IL1β and IL6 in both AIH and health, the increase was more notable in AIH patients. IL1β and IL6 increased the frequency of $CD39^{pos}$ Tregs expressing CD127 in AIH patients but not HS.
Conclusion: Compared to health, Tregs in AIH display lower CD39 expression and are more prone to activation upon exposure to pro-inflammatory stimuli, indicating reduced phenotypic stability. These characteristics may contribute to impaired Treg suppressive function in AIH.

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Reduced expression of Tim-3 renders Th1 and Th17 effector cells less amenable to T-reg control in autoimmune hepatitis

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Background: In autoimmune hepatitis (AIH), CD4 effector immune-responses are permitted by defective $CD4^{pos}CD25^{pos}$ T-regs. In murine studies, apoptosis of Th1 effectors is mediated by binding of T-cell-immunoglobulin-and-mucin-domain3 (Tim-3) on their surface to Galectin-9 (Gal9) expressed by T-regs.
Aims: To test the frequency of $Tim\text{-}3^{pos}$ cells within the Th1 and Th17 subsets and to evaluate whether Tim-3 expression by CD4 effectors affects their responsiveness to T-reg control.
Methods: Thirty-nine AIH patients and 16 healthy subjects (HS) were studied. Frequency of cells positive for CD4, CD25, Tim-3, T-bet, RORC, IFN-g and IL-17, was assessed by cytofluorimetry. Proliferation of $CD25^{neg}$, $CD25^{neg}Tim\text{-}3^{pos}$ and $CD25^{neg}Tim\text{-}3^{neg}$ target cells was assessed by ^{3}H-thymidine incorporation after 5-day culture in the absence and presence of $CD4^{pos}CD25^{pos}CD127^{neg}$T-regs.
Results: The frequency of $Tim\text{-}3^{pos}$cells within the Th1 and the Th17 subsets was lower in AIH than in HS (Th1: $P < 0.001$; Th17: $P = 0.02$). In AIH, the frequency of $Tim\text{-}3^{pos}$cells correlated inversely with transaminase and $CD25^{neg}T\text{-}bet^{pos}$cell values. $CD25^{neg}$cells proliferated less than $Tim\text{-}3^{neg}$ and more than $Tim\text{-}3^{pos}$cells. Addition of T-regs reduced cell proliferation by 26% in AIH and by 53% in HS when undivided $CD25^{neg}$cells were used as targets; by 23% and by 25% when $Tim\text{-}3^{neg}$cells were the targets and by 47% and by 62% when the targets were $Tim\text{-}3^{pos}$cells.
Conclusion: Compared to undivided $CD25^{neg}$ and $Tim\text{-}3^{neg}$, $Tim\text{-}3^{pos}$cells proliferate less vigorously and are more susceptible to T-reg control. In AIH, down-regulation of Tim-3 renders Th1 and Th17 CD4 effectors less amenable to immune-regulation and therefore more likely to inflict and perpetuate liver damage.

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Defective T-regulatory function in autoimmune hepatitis may partially derive from a pro-inflammatory skewing of $Gal9^+$ T-regs

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Background: In autoimmune hepatitis (AIH) $CD4^{pos}CD25^{pos}$ regulatory T-cells (T-regs) are defective in their ability to control CD4 T-cell effector function. T-regs express Galectin9 (Gal9), a b-galactosidase-binding-protein that inhibits Th1-mediated immune-responses by binding the T-cell-immunoglobulin-and-mucin-domain3 (Tim-3) on CD4 effector cells. In AIH T-regs express reduced levels of Gal9.
Aims: To characterise transcription factor and cytokine profiles of peripheral-blood-derived $Gal9^{pos}$T-regs.
Methods: Thirty-four ANA/SMA+ patients and 17 healthy subjects (HS) were studied. Expression of CD4, CD25, CD127, and Gal9, and the transcription factor and cytokine profile of T-regs were determined by cytofluorimetry. T-reg suppressor function was evaluated in a proliferation assay following co-culture with $CD25^{neg}Tim\text{-}3^{pos}$ and $CD25^{neg}Tim\text{-}3^{neg}$ autologous target cells.
Results: Within $Gal9^{pos}$cells the frequency of: (i) $FOXP3^{pos}$cells was lower in AIH than HS ($P < 0.001$); (ii) $T\text{-}bet^{pos}$, $GATA3^{pos}$ and $RORC^{pos}$cells was similar in AIH and HS; (iii) IL-10-producing cells was lower in AIH than in HS ($P < 0.001$) but higher than in the $Gal9^{neg}$ T-reg fraction for both (AIH: $P = 0.001$; HS: $P < 0.001$); (iv) TGF-b-producing cells was lower in AIH than in HS ($P = 0.04$); (v) IFNg- and IL-17-producing cells was higher in AIH than in HS ($P = 0.002$ for both). Treatment with anti-IL-10 neutralizing antibodies reduced T-reg ability to suppress $CD25^{neg}Tim\text{-}3^{pos}$cell proliferation, while did not affect $CD25^{neg}Tim\text{-}3^{neg}$cell proliferation.
Conclusion: A skewing towards a pro-inflammatory phenotype and a reduced proportion of $FOXP3^{pos}$ and IL-10-producing cells within $Gal9^{pos}$T-regs may contribute to defective immunoregulation in AIH. The reduction of $Gal9^{pos}$T-reg suppression following anti-IL-10 blockade, suggests a role for IL-10 in $Gal9^{pos}$T-cell immune-regulatory function.

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FOXP3 and TGF-beta gene polymorphisms in allergic rhinitis

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Regulatory $CD4^+$ T (Treg) cells are effective cells in balancing immune responses to maintain immune tolerance. In this study, we hypothesized single nucleotide polymorphisms (SNPs) in genes involved in the development and function of regulatory T cells that related with susceptibility to Allergic rhinitis (AR). The role of Transforming Growth Factor Beta-1 (TGF-β1) at codons 10, 25 and Fork head box protein 3 (FOXP3) at position −3279 A>C and −924 A>G was evaluated in AR patients in comparison with controls. In a case-control study, 155 AR patients and 163 allergy-free controls were genotyped by using polymerase chain reaction sequence-specific primer (PCR-SSP) techniques. Examination of these SNPs showed that haplotype formed by FOXP3 −3279 A allele occurred significantly more frequent in patients than controls (odds ratio 1.44, 95% CI 1.312−2.66; $P = 0.001$). Our results suggested that the genes involved in the growth and development of regulatory T cells, specifically FoxP3, associated with susceptibility to AR.

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The role of the transcription factor E4BP4 in chronic *Trichuris muris* infection

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E4BP4 is a transcription factor which has recently been described to play an important role in the plasticity of leukocyte development, including in NK cells and CD4 T cells. In particular, it has been shown to play a role in the regulation and secretion of type 2 cytokines from CD4 T cells. The study of *Trichuris muris* infection in the mouse provides an excellent model for human intestinal helminth infection and inflammation of the large intestine. It is known that initiation of a Th2 response is required for parasite expulsion. Here we show, for the first time, that ablation of the transcription factor E4BP4 is sufficient to lead to the expulsion of the parasite in otherwise susceptible mice and that deletion of E4BP4 is associated with elevated Th2 cytokines including IL-13, IL-4 and IL-9. We thus conclude that E4BP4 plays an important role in regulating Th2 responses *in vivo*. Ongoing work will identify the specific cell populations involved.

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Intestinal homeostatic signals are lost in affected areas of ulcerative colitis patients inducing an abnormal skin homing phenotype in dendritic cells and T-cells they stimulate

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Background: Ulcerative colitis (UC) is considered a TH2 disease mediated by IL-13. Dendritic cells (DC) control the type and place of immune responses. We hypothesised that local factors controlling intestinal homeostasis in UC patients are either lost or masked by ongoing inflammation in inflamed areas of the gut.
Methods: Colonic biopsies from inflamed and non-inflamed areas of UC patients were cultured *in vitro*. Cytokine secretion in culture supernatants was determined. Cell-free supernatants were used to condition human blood enriched DC from healthy volunteers. Phenotype and function of DC was determined by flow cytometry and mixed leukocyte reactions respectively.
Results: Inflamed areas of the gut from UC patients had increased production of soluble pro-inflammatory cytokines resembling a TH1 profile. Levels of IL-13 were below the detection limit in most cases while IL-6 was the predominant secreted cytokine. DC conditioned with unaffected areas of UC patients acquired a regulatory 'gut-like' phenotype. However, DC conditioned with inflamed areas acquired a pro-inflammatory phenotype (determined as increased surface expression of HLA-DR, increased ongoing production of intracellular IL-6, IL-12 and decreased ongoing production of IL-10), increased expression of skin homing CCR8, did not decrease their stimulatory capacity for T-cells and primed them with the skin-homing CLA molecule. Such effects were abrogated with blocking anti IL-6, and mimicked in healthy areas exposed to IL-6 following *in vitro* culture.
Conclusion: Homeostatic signals of the gut are lost in affected areas of UC patients driving DC, through an IL-6 dependent mechanism, into a pro-inflammatory not gut-restricted phenotype.

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Invariant natural killer T cells in lesional skin of lupus erythematosus patients

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Lupus erythematosus (LE) is an autoimmune disorder associated with exclusive skin lesions (cutaneous LE, CLE) or visceral involvement (systemic LE, SLE). Patients with active SLE display numerically reduced *i*NKT cells [a unique subset of T lymphocytes characterized by an invariant T-cell receptor (*i*TCR) α-chain] in peripheral blood (PB).

Here, we investigated if this reduction in patients with active SLE may be the result of an accumulation at the site of inflammation. The frequency of *i*NKT cells in skin biopsies from patients with CLE ($n = 6$) and SLE ($n = 5$) was assessed by immunofluorescence using *i*TCR-specific antibodies. Biopsies from healthy individuals ($n = 6$) served as controls. In comparison, *i*NKT cell frequencies in PB were analysed in SLE ($n = 24$), and CLE patients ($n = 13$), and healthy controls ($n = 29$).

Quantitative analysis of skin biopsies revealed an enrichment of *i*NKT cells in SLE and CLE lesional skin (mean 4.6 and 4.5 cells/mm^2, respectively) compared to healthy skin (mean 1.9). The *i*NKT cells were located within the epidermis, in perivascular infiltrates or skin appendages. In contrast, the frequency of *i*NKT cells in PB was significantly diminished in SLE and CLE (mean ≤ 0.03%), as compared to 0.07% for controls.

In summary, we demonstrate that *i*NKT cells are present in skin lesions of CLE and SLE although they make up only a small proportion of infiltrating T-lymphocytes. This migration to the site of inflammation may explain the low frequency of *i*NKT cells in PB and suggests a potential pathogenic role for *i*NKT cells in different subtypes of lupus erythematosus.

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Looking for susceptibility factors in patients with cutaneous leishmaniasis from Tabasco, Mexico

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In Mexico the most frequent clinical form of leishmaniasis is called Localized Cutaneous Leishmaniasis (LCL), caused by *Leishmania mexicana*. Yet the same parasite also produces the more severe anergic form called Diffuse Cutaneous Leishmaniasis (DCL), where the parasite spreads out of control, causing severe mutilation and eventually invading the oral and nasopharingeal mucosae. Our group has found that *L. mexicana* lipophosphoglycan (LPG) activates cells of the innate immune system by binding TLR2. In healthy controls and LCL patients, NK cells stimulated with LPG led to cytokine production, whereas in DCL patients, the cytokine production in response to the LPG stimulus was reduced. Little is known about participation of the innate immune response and inflammation in the disease outcome. Several single nucleotide polymorphisms (SNPs) associated with this disease have been reported (as TNFα, IFNγ, IFNγR, IL-6, IL-4). But none of them had been analyzed in Mexican-Mestizo population. We have analyzed 12 SNPs from nine genes involved in immune responses. We found that polymorphism in the gene encoding IL-1β (−511 C/T) represents a variable influencing risk to a more severe form of the disease for patients infected with *L. mexicana*. We also analyzed IL-1β cytokine production in monocytes and expression in serum and in skin tissues, where we found an over expression. Additionally, we analized the pattern of genes within NK cells of patients with LCL versus DCL and controls. We compared the protein pattern of 2-D gels using serum from patients with LCL, DCL and controls.

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Occurrence of opportunistic infections in people living with HIV/AIDS following antiretroviral therapy in West Bengal, India

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Occurrence of opportunistic infections (OIs) is the main cause of morbidity and mortality in HIV infected patients. HIV makes the infected person immunocompromised by destroying his CD4 T-lymphocytes. When the CD4 count of a HIV infected person decreases he or she becomes susceptible to OIs. Antiretroviral Therapy (ART) decreases multiplication HIV. We followed 88 patients for 3 years, who were getting first line ART at CSTM. For analysis we divided 88 patients according to their sex. In each group we monitored ocurrence of OIs, increment of CD4 count. We found that respiratory tract infection (RTI) was the most frequently occurring infection in our study groups. Next common OIs were tuberculosis and oral candidiasis (OC). After following up for 3 years we noticed a significant decrease in OIs. Incidence of RTI in male in 1st year (71%) and in 3rd year (18%), ($P < 0.00001$), for males OC decreased from (20%) to (0%) ($P < 0.0002$). For make, EPTB/PTB decreased from (30%) to (2%) where ($P < 0.00002$). Similarly for the female incidence of RTI was (38%) which became (20%) in 3rd year ($P < 0.15$). OC (24%) decreased into (6%) in 3rd year ($P < 0.07$). In females EPTB/PTB dropped from (31%) to (3%) ($P < 0.005$). Study based upon the OIs we found occurrence of RTI (60% in 1st year and 19% in 3rd year), EPTB/PTB (30% in 1st year and 2% in 3rd year) and OC (21−2% in 3rd year) decreases significantly ($P < 0.0000004$), ($P < 0.0000004$) and ($P < 0.00008$) respectively.

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Infiltrating renal T cells are activated and respond to self antigen

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Around one in 10 people in developed countries suffer from Chronic Kidney Disease (CKD). CKD is a significant public health problem, affecting the quality of life of patients and putting strain on the health care service. Patients with progressive renal function loss are at greatest risk. This progressive renal injury may in part be mediated by the immune system, including activation of both lymphocytes and macrophages. These cell types are a major component of the interstitial infiltrate characteristic of progressive kidney disease.

We examined the T cell response to a sterile model of renal injury, unilateral ureteric obstruction (UUO) and found evidence of T cell proliferation and activation in the injured kidney. T cell receptor (TCR) Vb gene usage analysis and TCR sequencings identified multiple copies of the same sequence from the UUO kidney at day 7 and 14 post UUO with evidence of a dominant sequence occurring with high frequency indicating clonal T cell expansion. To identify the antigen responsible we used thymidine incorporation assays and were able to show that splenocytes from UUO mice proliferate in the presence of whole kidney extract as a source of antigen. Furthermore, preliminary data shows blockage of the MHC class II using blocking antibodies reduces this proliferation to background levels confirming that the response seen is due to antigen recognition.

This data suggests that in a sterile model of renal injury there is loss of tolerance to self-antigens. This represents a new mechanism for progressive injury to the kidney.

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Cancer cell surface coagulation kinetics depends upon tissue factor expression levels

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The association between malignancy and thrombosis is well established. The incidence of venous thromboembotic events (VTE) varies across different tumour types, with pancreatic cancer acknowledged as high risk. This increased risk is thought to be tumour driven and associated with tumour expression of tissue factor (TF) and tumour-derived microparticles. In this study cancer cell lines from phenotypically distinct tumours were assessed for cell surface TF expression and procoagulant potential was determined by a prothrombin time (PT) assay. Breast (T47D, MCF-7), colorectal (Colo320 and LoVo), head and neck (USSC 11b, 12, 81b and SIHN-011A) and pancreatic tumour cell lines (ASPC-1 and CFPAC-1) were assessed for TF expression by flow cytometry. A logarithmic relationship was established between clotting time (CT) and cell number that was consistent across all cell lines. Single cell PT was determined for each cell line from the slope of a logarithmically transformed data plot. A near linear relationship was observed between TF expression and single cell CT where a higher expression of TF resulted in a proportionally faster PT ($P < 0.001$). This study shows that a consistent relationship is observed between procoagulant potential and both cell number and TF cell surface expression for a range of malignancies.

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Duramycin demonstrates anti-proliferative properties and induces apoptosis in pancreatic cancer cell lines

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Duramycin, a 19 amino acid polypeptide containing lanthionine produced by *Streptoverticillium cinnamoneus* exhibits week antimicrobial properties against Gram-positive organisms. It binds to phosphatidylethanolamine (PE) on cell surfaces with high affinity and has been shown to disrupt the tumour cell surface based coagulation. The aim of this study was to assess the efficacy of duramycin on tumour cell proliferation and cell viability. Pancreatic cancer cell lines (MIA-PaCa-2, ASPC-1 and CFPAC-1) were assessed for cell surface expression of PE by flow cytometry. Procoagulant potential was determined by a prothrombin time (PT) assay. All tumour cell lines were shown to express cell surface PE. Duramycin significantly inhibited the proliferation of pancreatic cancer cells in a dose dependent manner and the percentage of apoptotic cells increased in a dose-dependent manner, with the addition of duramycin. Furthermore, blocking of PE by duramycin resulted in a slower CT for ASPC-1 and CFPAC-1 cells. In summary, duramycin was shown to reduce cell proliferation, induce apoptosis and disrupt cell surface supported coagulation in pancreatic cancer cells.

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Understanding the dual role of murine $\gamma\delta$ T cells in tumour surveillance and tumour progression

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During the last 15 years, $\gamma\delta$ T lymphocytes have been described to mediate immune surveillance against tumour development. The anti-tumour function of $\gamma\delta$ T cells stems from their potent cytotoxicity and their ability to produce high amounts of IFN-γ. However, following the discovery of their ability to produce IL-17A, $\gamma\delta$ T cells were recently implicated in the promotion of tumour development. Indeed, IL-17A itself has been paradoxically associated with both pro- and anti-tumour effects. Building on this debate, we aim to understand the dual behaviour of murine $\gamma\delta$ T cells in tumour surveillance versus tumour progression. Our models are two transplantable tumour mouse cell lines; B16 and ID8, reported as having better or worse growth characteristics in the absence of $\gamma\delta$ T cells when compared to controls, respectively. We are first interchanging the route of injection of both models; sub-cutaneous versus intraperitoneal, in order to study the impact of the tumour site. We will then characterize the tumour microenvironment and the phenotype and functional properties of distinct subsets of tumour-infiltrating $\gamma\delta$ T cells in each model. We expect this project to make a significant contribution to our understanding of the cellular and molecular mechanisms that underlie $\gamma\delta$ T cell responses to tumours.

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The Th1, Th2, Th17 and regulatory T cells balance in lean and obese individuals with or without diabetes

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Obesity is associated with adipose tissue inflammation that is involved in the development of insulin resistance and type 2 diabetes. The main source of inflammatory mediators in obese adipose tissue is macrophages. Recent animal studies suggest that Th1 and $CD8^+$ cytotoxic T cells are detrimentally involved in the attraction and differentiation of adipose tissue macrophages, whereas Th2 and, predominantly, regulatory T cells (Tregs) act protective. IL-17, a pro-inflammatory cytokine that is produced by several cell types, including Th17 cells, is often associated with diseases that are characterised by tissue inflammation. However, the role of these immune cell types in obesity-associated adipose tissue inflammation has not been well-defined in human. Therefore, we aimed in the present work to compare the different immune cell types in the peripheral blood and adipose tissue of lean and obese individuals with and without diabetes.

Peripheral blood mononuclear cells and adipose tissue biopsies were isolated from adult lean and obese subjects with and without diabetes and immunological parameters were assessed.

Data on the imbalance of these immune cell types between lean and obese subjects will be presented together with data on the level of expression of specific markers as well as inflammatory and anti-inflammatory cytokine and biochemical profiles. The data presented will generate the basic information required for developing an immunodiagnostic and/or immunotherapeutic strategy aimed at prevention/treatment of type 2 diabetes.

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Th17 associated proteins in Juvenile systemic lupus erythematosus

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The pathogenesis of adult-onset systemic lupus erythematosus (SLE) has been linked to Th17 cells due to reported increased serum IL-17. There have been few investigations into the presence and role of Th17 cells within the more severe, juvenile-onset SLE (JSLE) phenotype. Therefore the objective of this study was to investigate the expression of Th17-associated cytokines and transcription factors within JSLE and control patients.

PBMC RNA was extracted from JSLE ($n = 11$) and control ($n = 10$) patients. RT-PCR quantified levels (mean ± SEM) of IL-17A, IL-23, IL-23R and RORC mRNA relative to RPL13A housekeeping gene. Plasma from JSLE ($n = 20$) and control ($n = 19$) were analysed for levels of IL-23 (mean ± SEM); results were correlated to disease activity markers. Plasma from JSLE ($n = 11$) and control ($n = 5$) were analysed for IL-17 levels (mean ± SEM).

IL-17A and IL-23 mRNA expression was significantly higher in JSLE PBMCs compared to controls (IL-17A: 0.30 (±0.08) versus 0.07 (±0.02) $P = 0.018$; IL-23: 0.41 (±0.11) versus 0.34 (±0.254), $P = 0.042$). RORC and IL-23R mRNA expression was also raised in JSLE compared to controls, although not statistically (IL-23R: 0.43 (±0.16) versus 0.16 (±0.07), $P = 0.189$; RORC: 0.52 (±0.16) versus 0.3 (±0.13), $P = 0.218$). IL-17A was undetectable in JSLE and control plasma. IL-23 was increased in JSLE plasma (2437 ± 778 pg/ml) compared to controls (1417 ± 365 pg/ml); this did not correlate with disease activity markers.

These results indicate Th17 cells may be playing a pathogenic role in JSLE. The inability to find IL-17 in JSLE patient plasma suggests it may be expressed at site specific locations i.e. the kidneys. The higher level of IL-23 mRNA and protein in JSLE patients indicates the presence of the pro-inflammatory environment required for Th17 differentiation.

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Effect of cytokine-induced STAT1 signaling on STAT3 activation and binding

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Background: Chronic mucocutaneous candidiasis (CMC) is a primary immune deficiency characterized by susceptibility to Candida infection of skin, nails and mucous membranes. We previously reported that CMC patients with autosomal-dominant CMC and hypothyroidism have defective Th-17 responses (Ng et al., JACI 2010) even though they do not have a mutation in the STAT3 gene. We have recently reported (van de Veerdonk et al., NEJM 2011) that these patients have a gain-of-function mutation in the CC region of the signal transducer and activator of transcription (STAT)-1 gene leading to hyperphosphorylation of STAT1 and subsequent over-expression of STAT1 activated genes. However, how this leads to decreased IL-17 production is unknown. In this study we assess the effect of STAT1 activation on STAT3 and STAT1/STAT3 heterodimers binding to the high affinity serum-inducible element (hSIE) as well as a STAT-binding element within the IL-17 promoter.
Methods: Assessment of STAT1 and STAT3 activation was performed by detection of phosphorylated proteins using western blotting. DNA binding was investigated using the electrophoretic mobility shift assay (EMSA) to assess binding to hSIE and IL-17 promoter sequences following activation of STAT1 and STAT3 by relevant cytokines (IFN-gamma and IL-23) in peripheral blood mononuclear cells (PBMCs) or EBV transformed cells lines.
Results: We demonstrate that activation of PBMCs with specific cytokines has a differential effect on the activation of STAT1 and STAT3 signaling pathways, interactions and DNA binding demonstrating that alterations of STAT1 function such as hyperactivity can interfere with and modify functions of STAT pathways.

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Investigation of the role of B cells as antigen presenting cells in primary biliary cirrhosis

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Primary Biliary Cirrhosis (PBC) is an autoimmune, chronic liver disease characterized by breakdown of tolerance against the ubiquitously expressed, mitochondrial autoantigen pyruvate dehydrogenase complex (PDC). In particular, patients with PBC exhibit high titre, anti-mitochondrial autoantibodies (AMA) against PDC-E2 component, although cell damage is restricted to biliary epithelial cells (BEC).

As PDC-E2 has been demonstrated to relocate to the cell surface of BECs during disease, the aim of this study is to explain the paradoxical disease specificity by testing the hypothesis that autoreactive PDC-E2-specific B cells, expressing membrane bound BCR, act as antigen presenting cells acquiring relocated PDC-E2 leading to the activation of autoreactive CD4 T cells.

We have isolated cDNAs encoding both heavy and light chain immunoglobulin variable regions reactive against PDC-E2. These were used to construct plasmids containing chimeric PDC-E2-binding BCR, which were then introduced into suitable B cell lines.

To isolate these cDNAs two complementary approaches were taken. The first was to isolate cDNAs from newly generated B cell hybridomas secreting anti-PDC monoclonal antibodies.

The second approach, involved the synthesis of previously documented cDNAs encoding anti-PDC immunoglobulin variable regions. Using this approach we report the construction of plasmids encoding chimeric PDC-specific BCR and the successful generation of B cells expressing membrane bound PDC-specific BCR for the first time.

The generation of B cells expressing membrane bound PDC-specific BCR will allow us to examine the acquisition of relocated PDC from biliary epithelial cells and the subsequent activation of anti-PDC specific CD4 T cells.

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The important role of dendritic cells in innate immune response against Friend virus induced immunsupression and tumorigenesis

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Scientific knowledge of retrovirus infections is relatively well established, but the mechanisms of retrovirus-associated immune suppression are still not understood. Friend murine leukemia Virus (FV) infection of immunocompent mice is a well established model to acquire further knowledge about these suppression mechanisms. Interestingly, BALB/c mice are infected by low doses of virus and die from FV-induced erythroleukemia, while C57/BL6 mice are recover from infection. Due to the central role of dendritic cells (DCs) in the induction of anti-viral responses, we asked for their functional role in the genotype-dependent sensitivity towards FV infection. Our previous studies have shown that FV-infected DCs induced a regulatory phenotype and suppressive function in cocultured DO 11.10 $CD4^+$ T cells (Treg).

Here we show that bone marrow-derived DCs derived from FV-infected mice of BALB/c and C57/BL6 genotype showed increased endocytotic activity and lower expression of costimulatory receptors as compared with DCs derived from uninfected mice. FV-infected DCs are poor T cell stimulators *in vitro* and *in vivo*. In order to identify key molecules that contribute to the FV-induced alterations in DC function, the expression pattern of cytoplasmatic proteins in uninfected and FV-infected DCs from both mice strains was analyzed by protein-mass fingerprinting. By that approach numerous proteins were identified as differentially expressed.

Ongoing work is focussed on elucidating the functional role of proteins which are differentially expressed in FV-infected DCs in a genotype-dependent manner and may contribute to the differential course of FV infection *in vivo* in BALB/c versus C57/BL6 mice.

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Differences in M1 and M2 macrophage distribution between symptomatic carotid artery and femoral artery plaques

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Macrophages within atherosclerotic plaques have traditionally been linked to plaque instability, however, recent interest has focussed on the heterogeneous nature of plaque macrophages. The aim of this study was to compare differences in macrophage heterogeneity and morphological composition between atherosclerotic plaques isolated from distinct vascular beds, specifically from recently symptomatic patients with carotid disease and femoral plaques from patients with severe limb ischemia. Plaques were obtained from 32 patients undergoing carotid endarterectomy and 25 patients undergoing common femoral endarterectomy or lower limb bypass. Carotid artery plaques had greater numbers per plaque area of macrophages and T cells ($P < 0.001$) consistent with a more inflammatory nature. Critically, the proportion displaying pro-inflammatory M1-macrophage activation markers, iNOS, MHC class II and SOCS3 was significantly increased in the carotid compared to femoral plaques ($P < 0.001$). By contrast, femoral plaques displayed greater proportions of M2-macrophage markers, dectin-1, SOCS1, and CD163 ($P < 0.001$). Morphometric analysis demonstrated carotid plaques had significantly increased percentage areas of lipid and confluent leukocytic infiltrates. Areas of fibroconnective tissue were significantly greater in femoral plaques and percentage area of confluent calcification and collagen was elevated. In conclusion, plaques from distinct vascular beds show distinct morphology and M1/M2 macrophage distribution. Carotid plaques exhibit more M1-macrophages and an inflammatory, lipid-rich phenotype both of which have been associated with plaque instability. By contrast femoral plaques have a predominance of M2-cells, and a comparatively more stable morphology. This study suggests a role for developing novel, more specific drug therapies which target M1-macrophages in symptomatic carotid disease.

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A role for tenascin-C in driving Th17 cell polarization in the RA joint?

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Rheumatoid arthritis is characterized by persistent synovial inflammation and progressive joint destruction, mediated by innate and adaptive immune responses. Cytokine blockade successfully treats some patient subsets, however approximately 50% do not respond to this approach. Targeting pathogenic T lymphocytes is emerging as an effective alternative/complementary therapeutic strategy. However, the factors that control T cell activation in joint disease are not well understood. Tenascin-C is an arthritogenic, extracellular matrix glycoprotein that is not expressed in healthy synovium but is elevated in the rheumatoid joint where high levels are produced by myeloid cells. Amongst these cells, tenascin-C expression is most highly induced in activated dendritic cells, prompting us to examine its role in this cell type. We found that dendritic cells derived from tenascin-C null mice produce lower levels of cytokines and exhibit specific defects in Th17 cell polarization, compared to wild type mice. Moreover, tenascin-C null mice display ablated IL-17 levels in the joint during experimental arthritis suggesting a key role in driving adaptive immunity in erosive joint disease.

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Does the suppressive activity of regulatory T cells differ between head and neck squamous cell carcinoma subsites and tumour stage?

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Head and neck squamous cell carcinoma (HNSCC) accounts for approximately 5% of all cancer incidences and is a term used to group together epithelial malignancies that develop from anatomically defined locations within the upper aerodigestive tract. The suppressive function of cell sorted, peripheral, regulatory T cells (Tregs; $CD4^+$ $CD25^{high/int}CD127^{low/-}$) from newly presenting patients with laryngopharynx ($n = 6$) or oropharynx ($n = 6$) tumours (stage T1; $n = 3$ and stage T4; $n = 3$ for each subsite), was investigated on autologous effector T cells ($CD4^+$ $CD25^-$) at a ratio of 1:1 using a 4 day CFSE proliferation assay. The percentage of suppression by both the $CD4^+$ $CD25^{high}CD127^{low/-}$ and the $CD4^+$ $CD25^{int}CD127^{low/-}$ Tregs from patients with T4 laryngopharynx tumours was greater than that induced by Tregs from patients with T1 laryngopharynx tumours, whereas the converse was generally true for Tregs from oropharynx patients. The Tregs from the laryngopharynx patients with stage T4 tumours tended to show greater suppression than those from patients with T4 oropharynx tumours, however Tregs from oropharynx patients with T1 tumours were more suppressive than the T1 laryngopharynx cohort. In addition, the Tregs with high CD25 expression were more suppressive than those with intermediate CD25 expression in the later stage tumours of either origin. Although these results did not reach significance in the current pilot study, it appears that both subsite and stage of the tumour influence the Treg activity in the periphery of HNSCC patients.

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Th17 cells activate NK cell mediated cytotoxicity to control tumour growth via IL-21

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The role of Th17 cells in the prevention or enhancement of anti-tumour immunity remains unclear, with evidence for both pro- and anti-tumour effects. We report here on a novel role for IL-21 derived from Th17 cells in the activation of tumour cell killing by NK cells. We observed that the growth rate of B16 tumours was enhanced in $IL\text{-}17^{-/-}$ mice, and this was associated with significantly reduced infiltration of NK cells. We found that Th17 cells indirectly promoted tumour cell cytotoxicity via the activation of NK cells. Although IL-17 was required for the activation of NK cells in co-culture experiments with Th17 cells, IL-17 did not directly activate NK cells. Furthermore, *in vitro* polarised Th17 cells did not mediate direct cytotoxicity but did activate NK cell killing of tumour cells in an IL-21 dependant manner. In addition, recombinant IL-21 enhanced expression of cytotoxic markers on NK cells and enhanced their ability to kill tumour cells *in vitro*. Using an immunotherapeutic approach involving a TLR agonist and PI3kinase inhibitor in the B16 melanoma model in mice, we found that successful tumor regression was associated with significantly increased numbers of T-cells co-producing IL-17 and IL-21, and with increased numbers of cytotoxic tumour infiltrating NK cells. Furthermore, infusion of recombinant IL-21 into tumours delayed tumour growth *in vivo* by increasing infiltration of NK cells into the tumour. These studies have revealed a novel role for Th17-derived IL-21 in control of tumour growth, which could be exploited for the rational design of anti-tumour therapies.

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The effects of acute exposure to cigarette smoke extract on T cell receptor signalling

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T cells are important in adaptive immunity to infection, but inappropriate signalling through the T cell receptor (TCR), combined with unwanted responses to self-antigens, promotes development of autoimmune inflammatory diseases. Cigarette smoking is a prevalent environmental risk factor for rheumatoid arthritis, among other inflammatory diseases. However, the mechanisms behind its contribution to disease processes are not understood. Thus we investigated cigarette smoke extract (CSE) as a potential trigger for alterations in TCR signalling, which could promote disease. CSE was generated from Marlboro Red cigarettes, and Jurkat T cells or peripheral blood mononuclear cells (PBMC's) were exposed to the diluted CSE for 24 h, before a range of TCR signalling parameters were measured. Exposure to CSE caused a reduction in global protein tyrosine phosphatase (PTP) activity, as well as reductions in the activity of specific PTPs, namely CD45 and Lyp. There was reduced signal transduction overall, as demonstrated by decreased Ca^{2+} mobilization in response to TCR activation. Ca^{2+} release independent of the TCR remained unaltered. The mechanism by which CSE exerts its effects could be oxidative, as exposure of cells resulted in depletion of the antioxidant glutathione. It is likely that soluble components and particulate matter (PM) play a role, as removal of PM reduced the potency of the CSE. Overall, acute exposure to CSE alters TCR activation, possibly through oxidation of key signalling proteins. This study shows that smoking can potentially alters T cell responses, which may contribute to impaired regulation of the immune system, thus promoting autoimmune inflammatory diseases.

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The effect of age on the mobilisation of human Langerhans' cells: an *in vitro* explant model

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With increasing age the immune system displays a functional decline. In the skin this is associated with an increased incidence of epidermal malignancies and infections. At skin surfaces epidermal Langerhans' cells (LC) are sentinels of the immune system. They migrate to draining lymph nodes where they present antigen to T lymphocytes. Langerhans' cell migration is orchestrated by two cytokines: interleukin (IL)-1β and tumour necrosis factor (TNF)-α. With increasing age LC numbers decline and *in vivo* migration is impaired secondary to reduced availability of IL-1β.

An epidermal explant model has been established to investigate further the effect of ageing on LC migration. Explants were prepared from skin of healthy young (≤30 years) and healthy aged volunteers (≥70 years), and cultured for 24 h in media alone, or media containing either IL-1β or TNF-α. Counts of LC were performed using fluorescence microscopy.

Baseline LC counts were reduced by 11% in aged volunteers ($P < 0.001$). At 24 h, LC had migrated spontaneously from explants of young volunteers. In contrast, there was little or no migration from explants of aged volunteers (mean young 16.6% migration, compared to mean aged 2.1% migration, $P < 0.0001$). Addition of IL-1β promoted migration (mean 13.0%) in aged volunteers. There was less migration in response to TNF-α (mean 7.1%).

This explant model provides further evidence that LC mobilisation is impaired in aged human skin and is a useful tool to study the mechanisms of human LC function *in vitro.*

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Alterations in OX40 and 4-1BB in $CD4^+$ $CD28^{null}$ T-cells identify a group of patients with coronary atherosclerosis that could benefit from targeted modulation of costimulatory pathways

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Background and purpose: T cells have pivotal roles in the immune response that drives atherosclerosis in patients with coronary artery disease (CAD). Of note, a peculiar subset of T cells, the $CD4^+CD28^{null}$ T lymphocytes, expand in CAD and their frequency correlates with clinical severity. $CD4^+CD28^{null}$ T cells are highly inflammatory and cytotoxic, in spite of lacking the costimulatory receptor CD28, which is crucial for optimal function of T lymphocytes. The mechanisms that govern the function of $CD4^+CD28^{null}$ T cells are not known. We investigated the expression and roles of alternative costimulatory receptors in $CD4^+CD28^{null}$ T cells from CAD patients.
Methods: The expression of alternative costimulatory receptors ICOS, CTLA-4, PD-1, OX40 and 4-1BB was quantified in T cells from peripheral blood and atherosclerotic tissue.
Results: We found that levels of costimulatory receptors OX40 and 4-1BB were significantly higher in circulating $CD4^+CD28^{null}$ T cells from patients with severe CAD (myocardial infarction) but not stable angina. Furthermore, we showed that $CD4^+CD28^{null}$ T cells represent an important proportion of $CD4^+$ T lymphocytes in atherosclerotic plaques and express OX40 and 4-1BB. Blockade of the alternative costimulatory receptors OX40 and 4-1BB reduced the ability of $CD4^+CD28^{null}$ T cells to produce IFN-g, TNF-a and perforin.
Conclusions: Costimulatory pathways are altered in $CD4^+$ $CD28^{null}$ T cells from patients with severe CAD. Blockade of OX40 and 4-1BB costimulatory receptors decreases the inflammatory and cytotoxic function of $CD4^+$ $CD28^{null}$ T cells. Modulation of costimulatory pathways will allow specific targeting of this cell subset and may improve the survival of ACS patients.

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Human dendritic cell deficiency is caused by *GATA-2* mutation

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The human syndrome of Dendritic Cell, Monocyte, B and NK Lymphoid (DCML) deficiency is associated with severe depletion of peripheral blood and dermal DCs but preservation of Langerhans cells and macrophages. Peripheral blood effector T cells are preserved but $CD4^+CD25^+Foxp3^+$ Treg cells are depleted. Serum Flt-3 ligand is elevated up to 100-fold. The stem cell compartment is abnormal with complete loss of the multilymphoid progenitor; a cell that gives rise to DC, monocytes and lymphocytes. Affected individuals have compromised immunity to mycobacteria, papillomaviruses and fungal pathogens and may develop autoimmunity. Seeking a genetic etiology, we sequenced the exomes of four subjects with DCML deficiency. Only one gene, *GATA-2*, was mutated in all subjects. Subject 1 had a frameshift at G200 while Subject 2 and 3 had T354M and R398W missense mutations, respectively. Subject 4 had a point mutation within a splice acceptor site. Lately we identified three more subjects: two with novel *GATA-2* mutations (S106 frameshift; R398Q) and another with the R398W mutation. We also confirmed the presence of the R398W mutation in deceased relatives of Subject 3 in keeping with autosomal dominant inheritance. As some mutations are likely to produce completely non-functional GATA-2, hemizygosity is probably sufficient to cause the DCML phenotype. GATA-2 is known to play a role in stem cell renewal but these studies show it is also required for the development of DCs and other mononuclear cells. *GATA-2* mutation defines a new haematopoietic disorder and is the most prevalent cause of DC deficiency in humans.

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Innate IL-17-secreting Vγ4 subset of $\gamma\delta$ T cells play a crucial role in the development of experimental autoimmune encephalomyelitis

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$\gamma\delta$ T cells are a key source of innate IL-17, with important roles in anti-bacterial and anti-fungal immunity. We have shown that IL-17-producing $\gamma\delta$ T cells play a critical role in the development of autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE). Furthermore, IL-17 production by peripheral $\gamma\delta$ T cells is dependent on IL-1 and IL-23 signalling. Here, we show that $\gamma\delta$ T cells derived from thymus can also produce IL-17 in response to IL-1 and IL-23, at comparable levels to lymph node (LN) derived $\gamma\delta$ T cells. Furthermore, IL-1 and IL-23 promote the expansion of a $CD44^+$ $CD25^+$ population of $\gamma\delta$ T cells in both the thymus and LN, of which >95% produce IL-17. The $CD44^+$ $CD25^+$ $\gamma\delta$ T cells were almost entirely of the Vγ4 subset. These Vγ4 T cells can be expanded in response to IL-1 stimulation and produce IL-17A, IL-17F and IL-22 in response to IL-1 and IL-23. Vγ4 T cells are present in the CNS and draining lymph nodes of mice with EAE at the height of disease, and are a major source of both IL-17 and IFN-γ. IL-1RI$^{-/-}$ mice are resistant to induction of EAE and this can be overcome by transfer of wild-type CD3 T cells. However depletion of Vγ4 from the transferred CD3 T cells significantly delayed the onset of EAE. Conversely, IL-1RI$^{-/-}$ developed EAE following transfer of purified Vγ4 cells. These data suggest a crucial role for IL-1RI expressing V$\gamma4^+$ $\gamma\delta$ T cells in the development of autoimmunity.

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Epstein-Barr virus (EBV) latent membrane protein 2A (LMP2A) enhances antigen presentation of B cells, possibly contributing to experimental autoimmune encephalomyelitis (EAE) severity

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Multiple Sclerosis (MS) is an inflammatory, autoimmune disease of the central nervous system. The cause of MS is still unknown but epidemiological and immunological studies have implicated Epstein–Barr Virus (EBV), which infects B cells, as a possible etiological agent involved in disease. Of particular interest is EBV Latent Membrane Protein 2A (LMP2A) due to our previous work revealing it as a potential contributor in autoimmunity. As B cells may be a key player in MS, we wanted to examine the role of LMP2A in the animal model Experimental Autoimmune Encephalomyelitis (EAE). Recombinant MOG protein was immunized into transgenic mice in which B cells express LMP2A. LMP2A mice show increased severity and incidence of disease, and faster onset of disease. This discrepancy cannot be explained by lymphocyte recruitment into the CNS or differences in serum antibody levels. Rather, LMP2A enhances antigen presentation to T cells and may play a role in early lymphocyte activation, setting the stage for an enhanced inflammatory environment.

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Oxygen level and hypoxia influence T cell polarisation

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Inflammatory environments such as the joint in rheumatoid arthritis are characteristically hypoxic in nature, profoundly affecting immune cell function. Innate immune cells are adapted to work in hypoxic environments and use anaerobic glycolysis for ATP production, but the effect of hypoxia on lymphocytes is less understood. We have investigated the effect of oxygen level on naïve $CD4^+$ T cell polarisation and signalling.

Cytokines associated with Th2 polarisation were increased at 8.5% oxygen but diminished at lower oxygen levels including constant 1% oxygen and 1% oxygen with reperfusion injury. T-bet, the transcription factor associated with a Th1 polarisation, was expressed at low oxygen levels and mildly expressed in cells cultured at 21% oxygen. GATA-3 expression, which is associated with Th2 polarisation, was found across many different oxygen levels, with expression increasing in stimulated PBMCs cultured at 1% constant oxygen, 8.5% oxygen (physiologically normal oxygen level) and 21% oxygen. Flow cytometry and western blot revealed altered Lck phosphorylation in Jurkats at different oxygen levels suggesting hypoxia may influence proximal signalling in T cells perhaps through modulation of the regulatory phosphatases, LYP and CD45.

These data suggest that hypoxia may alter the balance between Th1 and Th2 polarisation by a possible upregulation of transcription factors associated with Th1 that antagonise differentiation to a Th2. This may be partly due to altered proximal T cell signalling under hypoxia. We suggest this may have profound effects on the chronic hypoxic environment of chronic inflammatory environments such as rheumatoid arthritis.

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Evaluation of arginase, 5′-nucleotidase and lysozyme activity by monocytes from visceral leishmaniasis patients

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Visceral leishmaniasis (VL) is caused by *Leishmania donovani*, an obligate intracellular protozoan that parasitizes tissue macrophages. Intramacrophage infection by *L. donovani* results in potentially fatal visceral infections in man and the elimination of *Leishmania* parasites by the macrophage depends upon the mounting of an effective cell-mediated immune response by the mammalian host. There is a role of arginase in the immune response supporting its involvement in macrophage effector mechanism *in vitro* and *in vivo*. 5′-Nucleotidase, a plasma membrane component has been cited as a biochemical correlate of macrophage function in an altered morphological and biochemical state of activation and stimulation. The decrease in 5′-nucleotidase activity has been generally referred to as a characteristic marker of activated macrophages. Lysozyme is a secretary product of macrophage, which is constitutively produced. Lysosomal enzymes are involved in the defense functions of the body. In the present study, we have studied levels of arginase and 5′-nucleotidase (marker for macrophage activation) in monocytes of active VL patients and healthy controls. Lysozyme in culture supernatants collected from monocytes of active VL patients and healthy controls was also measured. We observed that the production of 5′-nucleotidase by cultured monocytes from active VL patients were significantly higher compared with the healthy controls. However, the levels of arginase and lysozyme by monocytes from VL patients were significantly low as compare to healthy controls. Our studies suggest that low levels of arginase and lysozyme and increased 5′-nucleotidase activity could be one of the mechanisms in the pathology of VL infection.

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Do activated monocytes impair regulatory T cell function in rheumatoid arthritis?

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Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting the joints. RA is associated with excessive immune activation, which may in part be due to a dysregulated function of regulatory T cells (Tregs). Our data show that the frequency of Tregs ($CD4^+CD25^+CD127^{lo}$) in peripheral blood (PB) of patients with inflammatory arthritis ($n = 7$ Psoriatic Arthritis and $n = 6$ RA) was slightly increased compared to healthy controls ($n = 9$) (6.90 ± 0.60% versus 5.75 ± 0.48%). Treg frequency was further significantly increased in the synovial fluid (SF) (15.89 ± 1.77%, $n = 11$, $P = 0.0001$). Surface marker staining of $CD14^+$ monocytes from PB and SF of these patients revealed a highly activated phenotype for SF monocytes (SFM) with increased expression of HLA-DR, CD54, CD40, CD86 and CD16. LPS-treated monocytes showed a similar phenotype to SFM and in co-culture, were able to increase the production of pro-inflammatory cytokines such as IL-17, IFNγ and TNF-α by $CD4^+CD25^+CD45RO^+CD127^{lo}$ Tregs relative to non-activated monocytes. This effect was shown to be partially mediated by soluble factors secreted by activated monocytes. Furthermore, Tregs in the presence of activated monocytes showed an impaired ability to suppress monocyte-derived TNF-α production. Our data suggest that despite an increase in Treg frequency in the rheumatic joint, their function may be subverted by activated monocytes.

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The influence of high molecular weight hyaluronan on CD4+ T lymphocytes in autoimmune patients

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It is well known that autoimmune diseases are characterized by breaking immune tolerance to self-antigens, while $CD4^+CD25^+$ regulatory T cells are dominant regulators of this process. The transcription factor FoxP3 is associated with regulation of Treg cell suppression activity, but the mechanism of FoxP3-mediated regulation is poorly understood. Recently up-regulation of Foxp3 by high m.w. hyaluronan in $CD4^+CD25^+$ regulatory T cells has been shown. The aim of the study is the estimation of high molecular weight hyaluronan influence on FoxP3 expression in CD4+ T cells of autoimmune patients. Peripheral blood samples were collected from seven healthy donors and seven autoimmune patients suffering rheumatoid arthritis. CD4+ T cells were negatively selected using the Treg isolation kit. Following 20-min incubation with hyaluronic acid two fractions hyaluronan-binding and non-binding CD4+ T cells were obtained.

We have established similar high level of intracellular FoxP3 in intact CD4+ T lymphocytes obtained both from healthy donors and patients. But we have observed the difference between H+ and H− fractions with regard to expression of CD25, CD39, IL-4 in healthy donors. Expression of these markers was significantly higher in the H+ fraction than in H− fraction. However these differences were not detected in RA patients.

FoxP3 level in CD4+ cells decreased in control group whereas this was not attributable to RA patients after 20-min incubation with hyaluronan. Downregulation of FoxP3 might be linked with CD4+ T cell activation. We suppose that hyaluronic acid can potentially differentiate functionally active regulatory T cells in health but not in RA.

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Systemic inflammation modulates Fc receptor expression on microglia in a mouse model of chronic neurodegeneration: implications for immunotherapy

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Chronic neurodegeneration is a world-wide health problem and it has been suggested that systemic inflammation can accelerate the onset and progression of clinical symptoms. A possible explanation is that systemic inflammation 'switches' the phenotype of microglia from a relatively benign to a highly aggressive and tissue damaging phenotype. We have investigated the molecular mechanism underlying this microglia phenotype 'switching' and demonstrate that mice with chronic neurodegeneration (ME7 prion model) show an increased expression of receptors that have a key role in macrophage activation and associated signalling pathways, including: TREM-2, Siglec-F, CD200R and IgG Fc receptors (FcγRs). Systemic inflammation induced by LPS further increased protein levels of the activating FcγRIII and FcγRIV, but not of other microglial receptors, including the inhibitory FcγRII. In addition to these changes in receptor expression, IgG levels in the brain parenchyma were raised during chronic neurodegeneration and these IgG levels further increased following systemic inflammation. Gamma-chain deficient mice show similar levels of IgG and microglial activation but modified pro-inflammatory cytokine expression in the brain following systemic inflammation. We conclude that systemic inflammation during chronic neurodegeneration increases the expression levels of activating FcγR on microglia, and thereby lowers the signalling threshold for antibody-mediated cell activation. At the same time, IgG influx into the brain could provide a cross-linking ligand resulting in excessive microglia activation that is detrimental to neurons already under threat by misfolded protein.

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Patients with complex regional pain syndrome have activating serum-autoantibodies against alpha 1 adrenoceptors and muscarinergic receptors

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Complex regional pain syndrome (CRPS) is a painful, usually post-traumatic condition in a limb. It is characterised by sympathetic, sensory, and motor dysfunction and skin abnormalities but the main symptom is pain. A range of treatments can enhance early recovery, but in patients who do not improve within 6 months, the disease typically has effects on longterm quality of life. Treatment of long-standing CRPS is empirical and often of limited efficacy. We have recently demonstrated that longstanding CRPS responds to treatment with intravenous immunoglobulin (IVIg). A response to IVIg often signals the importance of autoantibodies in disease. Therefore, we investigated whether CRPS sera contain autoantibodies against receptors which could explain both pain and autonomic signs. We purified IgG fractions from sera from patients and healthy controls, and investigated their effect on intracellular calcium and cell contraction in primary rat cardiomyocytes. We found abnormalities in both calcium handling and contractility in response to eight out of the 11 CRPS-IgG-preparations, but not in control-IgG incubated cells. Both baseline and transient calcium were reduced by 30% and 40% respectively and the myofibrillar sensitivity to calcium was increased 1.8-fold. The effects on intracellular calcium or myofibrillar sensitivity could be blocked by a muscarinergic receptor antagonist or an α1 adrenoceptor-antagonist respectively. Neither antagonist altered the depression of the calcium transient. Patch-clamping indicated that CRPS-IgG affects calcium channel function and increases the window current. We conclude that CRPS sera likely contain autoantibodies against both muscarinergic and alpha-1 adrenergic receptors which could explain important clinical observations.

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Obesity-associated aberrant inflammation does not influence experimental autoimmune myocarditis in C57BL/6J mice

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Obesity is reaching epidemic proportions worldwide and represents a new challenge for global health and wellbeing. Apart from the well charactarised comorbidities of diabeties and hypertension, obese individuals have alterations in basic immune function, including increased susceptibility to bacterial infections and autoimmune diseases. While the relationship between obesity and autoimmune diseases such as psoriasis and multiple sclerosis have been addressed, little is known about the effect of obesity on autoimmune myocarditis. We found mice fed a high-fat diet displayed increased cell proliferative capacity and elevated production of pro-inflammatory cytokines. In addition, obese OT-II mice displayed increased responses to OVA-peptide, demonstrating that the obese state alters antigen-specific CD4+ T cell immune responses. To address the impact of obesity on experimental autoimmune myocarditis (EAM), we immunized lean C57BL/6J mice normally resistant to EAM with myosin peptide ($MyHC_{614-629}$), as well as obese animals. In contrast to the susceptible Balb/c strain, neither lean nor obese C57BL/6J strain mice produced serum anti-MyHC IgG antibodies, nor displayed any increase in splenocyte proliferation or cytokine production in response to $MyHC_{614-629}$. Moreover, histological analysis revealed that obese C57BL/6J strain mice did not display the characteristic hallmarks of leukocyte infiltration or cardiac fibrosis clearly evident in the hearts of Balb/c mice following EAM. Therefore, despite the presence of pro-inflammatory cytokines known to promote myocarditis, obese C57BL/6J mice remain resistant to EAM. Taken together these data suggest that obesity *per se* does not adversely affect autoimmunity but rather may overtly influence pathology only in genetically susceptible individuals.

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Control of early cartilage destruction in inflammatory arthritis by death receptor 3

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Aims: Death Receptor 3 (DR3), the closest tumour necrosis factor receptor superfamily relative to TNFR1, is essential for the accumulation and function of effector T cells in multiple autoimmune and inflammatory disease models, and the development of bone erosions late in animal models of inflammatory arthritis. Here, we investigated the role of DR3 in cartilage destruction during early stages of disease.

Methods: DR3-deficient ($DR3^{KO}$) and $DR3^{WT}$ littermates were induced for antigen-induced arthritis (AIA) using methylated BSA. Joints were sectioned and analysed for cartilage destruction using histo- and immunohistochemistry at early (3 days) and late (21 days) timepoints after intra-joint mBSA challenge. MMP-9 ELISAs were performed for *in vitro* culture experiments.

Results: Resistance to cartilage destruction in $DR3^{KO}$ mice was observed even at early timepoints (17.3% versus 1.9%, $DR3^{WT}$ versus $DR3^{KO}$, respectively; $P = 0.03$), prior to the main accumulation of effector T cells and macrophages into the joint. $DR3^{KO}$ joints exhibited reduced levels of $Ly6G^+$ neutrophils (5.3% versus 1.3%, $DR3^{WT}$ versus $DR3^{KO}$, respectively; $P = 0.001$) and the cartilage-destroying enzyme, matrix metalloproteinase 9 (5.0% versus 2.5%, $DR3^{WT}$ versus $DR3^{KO}$, respectively; $P = 0.04$). *In vitro* experiments with human cells showed that TL1A, DR3's only confirmed ligand, did not trigger MMP-9 release, but neutrophils produced >350 times more MMP-9 on a per cell basis than macrophages or fibroblasts (253 500 versus 690 versus 80 pg/h/10^6 cells; neutrophils versus macrophages versus RA synovial fibroblasts, respectively).

Conclusions: DR3 controls early innate immune-driven development of cartilage destruction in inflammatory arthritis by regulating MMP-9 production and neutrophil accumulation.

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Interleukin-1 and platelets as key drivers of cerebrovascular inflammation

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The infiltration of neutrophils across vascular endothelium into tissue contributes to neurological disease, including in cerebral ischaemia. *In vitro* evidence suggests a key role for platelet-derived interleukin-1alpha (IL-1α) in mediating neutrophil migration across the cerebrovascular endothelium (1). This study investigates the role of platelets and IL-1 using *in vivo* models of vascular inflammation.

Three murine models of neutrophil migration were used. Bacterial endotoxin, lipopolysaccharide (LPS), was injected intraperitoneally or into a dorsal air pouch. Lavage of the respective cavities was performed 6 h later. LPS was stereotactically injected into the striatum, and the brain fixed and removed 24 h later. Flow cytometry or immunohistochemistry were used to assess migrated neutrophil numbers. To determine the role of platelets, platelet depletion was induced via an anti-CD41 antibody. To determine the role of IL-1, IL-1α/β knockout mice were used. Cytokines were quantified using cytometric bead array.

In all models, platelet depletion abolished the neutrophil migration, indicating a key role for platelets in this process. A robust inflammatory response was seen in serum cytokines after LPS injection and platelet depletion selectively abrogated the increase in serum IL-1α. IL-1α/β knockout mice revealed a significant difference in neutrophil migration between knockout animals and controls in the encephalitis model, with no difference in the peritonitis model.

In an *in vivo* peritonitis model, neutrophil migration appears to be dependent on platelets, yet independent of IL-1. In an encephalitis model, neutrophil migration appears dependent on platelets and IL-1, raising the possibility of tissue-specific inflammatory pathways.

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The role of host CD4 T cells in germinal centre mediated autoantibody production in GVH responses

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The class-switched and long-lasting nature of autoantibody responses suggests germinal centre (GC) output and a requirement for T follicular-helper (T_{FH}) cells. Here we examine a well-characterised model of GVH- induced humoral autoimmunity to delineate the respective roles of donor and recipient CD4 T cells in the development of autoreactive GCs.

Adoptive transfer of MHC class II disparate bm12 CD4 T cells into B6 mice resulted in production of anti-nuclear autoantibody with 60% (±11%) of the splenic B cell follicles exhibiting PNA^{+ve}/GL 7^{+ve} germinal centre morphology at week 7. In contrast bm12 CD4 T cells adoptively transferred into $TCR^{-/-}$ (T cell deficient) mice provoked a shorter-lasting autoantibody response, despite being initially as strong. Notably, B cell follicles did not differentiate into GCs and in keeping with an extra-follicular response, only $CXCR4^{hi}$, $ICOS^{hi}$ (and not $CXCR5^{hi}$) CD4 T helper cells were detectable. Confirmation that recipient CD4 T cells were required for GC development was provided by restoration of GC reactions upon transfer of additional WT B6 CD4 T cells into bm12 CD4 T cell-challenged $TCR^{-/-}$ mice (62 ± 4% of follicles).

Although the initiation of GVH-induced humoral autoimmunity is mediated by donor CD4 T cells, host CD4 T cells are nevertheless required for development of GC reaction and production of long lasting autoantibody. This mechanism may drive diversification of autoantibody responses.

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VEGF directly suppresses activation of T cells from ascites secondary to ovarian cancer via VEGF receptor type 2

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The role of vascular endothelial growth factor (VEGF) in tumor angiogenesis is well characterized; nevertheless, it is also a key element in promoting tumor evasion of the immune system by downregulating dendritic cell maturation and thus T cell activation. We have shown before that VEGF plays a suppressive role in the proliferation of T cells isolated from blood from ovarian cancer patients and healthy individuals. We sought to investigate the possible direct effect of VEGF on T cell activation and through which type of VEGF receptor (VEGFR) it exerts this effect in T cells isolated from ascites of ovarian cancer patients. T cells were expanded in cultures with anti-CD3 and IL-2 with or without VEGF for 14 days, and the number of T cells was assessed. Cultured T cells were also tested for their cytotoxic activity in a standard 4-h ^{51}Cr-release assay, and the expression of VEGFRs 1, 2, and 3 was assayed by flow cytometry, immunocytochemistry, and Western blotting. The addition of VEGF in cultures significantly reduced T cell proliferation in a dose-dependent manner. It was shown that $CD3^+$ T cells express VEGFR-2 on their surface upon activation. Experiments with anti-VEGFR-2 antibodies showed that the direct suppressive effect of VEGF on T cell proliferation is mediated by VEGFR-2. We also showed that VEGF significantly reduced the cytotoxic activity of T cells. Overall, our study shows that VEGF directly suppresses T cell activation via VEGF receptor type 2.

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Th17 cells expressing KIR3DL2 and enriched for gut homing markers are increased in ankylosing spondylitis

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Background: T helper 17 (Th17) cells are a subset of pro-inflammatory CD4 T cells implicated in the Spondyloarthritides (SpAs). Ankylosing Spondylitis (AS), the commonest spondyloarthropathy, is genetically associated with HLA-B27 and IL-23 receptor polymorphisms. We have shown KIR3DL2+ expressing CD4+ T cells are expanded in the peripheral blood of individuals with AS. Here we aim to characterize these cells further.
Methods: KIR3DL2+ CD4+ T cell phenotype was investigated by flow cytometry. Production of cytokines by PMA/ionomycin stimulated-PBMCs was investigated by intracellular cytokine staining (ICS). Cytokine production by α-CD3/28-stimulated FACS-sorted KIR3DL2+ and KIR3DL2− CD4 T cells was investigated by multiplex bead analysis. Expression of KIR3DL2+ on CD4+ T cells was investigated after SEB stimulation and cytokines were investigated by ELISA.
Results: KIR3DL2+ CD4+ T cells increased in peripheral blood of HLA-B27+ SpA patients were enriched for expression of Th17 phenotypic markers, IL23R and CCR6, and the gut-homing chemokine receptor, CCR9. KIR3DL2+ CD4+ T cells from AS patients produced significantly more IL-17 than KIR3DL2− CD4+ T cells and IL-17 levels significantly increased with the Th17 cytokines rIL-23 and IL-1. SEB activation increased the number of KIR3DL2+ cells and IL-17 production more in AS patients than controls.
Discussion: KIR3DL2+ CD4+ Th17 cells are expanded in patients with Spondyloarthritis. These cells constitute a large proportion of peripheral blood CD4+ T cell IL-23R expression and produce increased levels of IL-17, which is further increased by the presence of Th17 cytokines. Our findings link HLA-B27 with IL-17 production and suggest new therapeutic strategies in AS/SpA.

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Human Langerin + dendritic cells are independent of epithelial Langerhans cells; a novel perspective on Langerhans cell histiocytosis

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Langerhans Cell Histiocytosis (LCH) is rare but potentially fatal disease. LCH lesions are characterised by the accumulation of Lang+ cells (LCH cells) admixed with lymphocytes, eosinophils, neutrophils and macrophages, which can occur is single or multiple organs. Due to their langerin expression, which has been thought to be specific to Langerhans cells (LC), LCH cells have been presumed to originate from LC. However, demonstration of the accumulation of LCH cells in non-epithelial tissues raises the question of the true origin of these cells

Examining human tissues by flow cytometry and fluorescence microscopy, we show that Langerin-expressing dendritic cells (DC) are found in healthy human dermis, lung and liver. These cells differ phenotypically from LC in their surface antigen expression, intracellular langerin distribution and TLR profile. They are also distinct in their anatomical location, repopulation kinetics following stem cell transplantation, and their self-renewal capacity in the recently described human dendritic cell deficiencies. We also show that langerin expression can be induced *in vitro* on peripheral blood CD11c+DC in 12−18 h in the presence of an inflammatory cellular milieu or specific cytokine combinations, including GM-CSF and TNFa or TGFb. Furthermore, examining lesional material, we show that the phenotype of LCH cells, by mRNA and surface antigen expression, is at least as close to that of Langerin-expressing DC as LC.

These findings suggest that langerin expression may be triggered by the inflammatory components of the lesions and crucially changes the emphasis of LCH research and consequently the search for effective treatments.

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HD6: a novel monoclonal antibody that recognises a subset of HLA-B27 molecules strongly implicated in spondyloarthritis disease pathogenesis

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The MHC class I antigen HLA-B27 (B27) is strongly associated with development of a group of closely related arthritic diseases, collectively known as spondyloarthropathies, including Ankylosing Spondylitis (AS). Transgenic rats (TG) expressing both human B27 and b_2-microglobulin genes develop spontaneous inflammatory diseases similar to AS. The mechanism by which B27 confers this susceptibility is unclear. B27 forms both heterotrimers (B27HT) associated with peptide and b_2-microglobulin, and also peptide-free heavy chain homodimers ($B27_2$). A pathogenic role for $B27_2$ has been proposed. We have generated a novel monoclonal antibody to $B27_2$ using phage-display technology − HD6 − which binds to *in vitro* refolded $B27_2$ but not B27HT complexes by ELISA. Here, the surface recognition of HLA-B27 by this novel mAb is characterised by flow cytometry both in human HLA-B27 transfected cell lines and in splenocytes isolated from HLA-B27 TG rats. For further characterisation of the HD6 epitope, cells were also (i) treated with acid, or (ii) cultured with HLA-B27-binding or control peptides prior to FACS analysis. These data demonstrate HD6 binds to an epitope present on the surface of B27-transfected cells and on a number of cell subsets from diseased B27 TG rats, consistent with $B27_2$ expression and recognition at the cell surface. HD6 will be a powerful tool to address the potential pathogenic role of $B27_2$ in SpA and may additionally have therapeutic potential.

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IFN-γ and arginine metabolism during Behçet disease

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Behçet Disease (BD) is a systemic chronic inflammatory disorder with uncertain etiology. We previously showed a concomitant significant increase in IFN-γ, IL-4 and nitric oxide, product of NOS, during disease acute phase. However, L-arginine, the NOS substrate, is shared by the arginases which can be induced by IL-4. To evaluate the NOS and arginases activity and their regulation by IFN-γ during BD, 32 patients with BD and 22 control subjects were included in this study. Peripheral Blood Mononuclear cells (PBMC) were separated on histopaque (Sigma-Aldrich) and cultured either in MEM or DMEM complemented with 10% of FBS and antibiotics. Cultures were performed either with or without control or patients' plasmas and subsequent treatment with either anti- IFN-γ, LNMMA or BH4 (Sigma-Aldrich). Culture supernatants were harvested after 24 h of incubation. NO and urea measurements were performed by modified Griess and Berthelot methods respectively.

Results: In all cases, NO levels in MEM were higher than those obtained in DMEM ($P < 0.05$). Our results showed a significant increase in NO production after culture treatment with patients' plasmas alone or with BH4 ($P < 0.05$). Culture treatment with anti-IFN-γ or LNMMA reduced significantly NO levels in culture supernatants. Urea production profile was inversed when compared to NO production. The treatment effect was also more significant in DMEM than in MEM. In conclusion, our results suggest that IFN-γ is implicated in NO production during BD. However, it seems that the NOS/arginase balance is more regulated at the post traductional level by a substrate competition.

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Deregulated apoptosis pathways in $CD4^+CD28^{null}$ T cells from patients with myocardial infarction

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Background: T lymphocytes, the main effectors of adaptive immunity, have key roles in the development and progression of atherosclerosis. The frequency of the $CD4^+CD28^{null}$ T cell subset increases significantly in patients with myocardial infarction. These cells, which characteristically do not express the CD28 costimulatory receptor, have been suggested to mediate plaque instability and recurrence of myocardial infarction.

Aim: Our aim was to investigate the mechanisms that lead to the accumulation of $CD4^+CD28^{null}$ T cells in patients with myocardial infarction, with the main focus on apoptosis pathways in these cells.

Methods: $CD4^+CD28^{null}$ T cells from peripheral blood of myocardial infarction patients and controls were tested for the expression of death receptors (Fas) and ligands (FasL), as well as the levels of anti-apoptotic (Bcl-2, Bcl-xL, survivin) and pro-apoptotic (Bax, Bim) proteins, using flow cytometry. In addition, analysis of apoptosis with Annexin V and 7-AAD as well as detection of activated caspase-3 was performed following *in vitro* activation of T cells.

Results: We found that $CD4^+CD28^{null}$ T cells express lower levels of anti-apoptotic proteins Bcl-2, Bcl-xL and survivin when compared to conventional $CD4^+CD28^+$ T cells. Furthermore, the pro-apoptotic protein Bax was increased in $CD4^+CD28^{null}$ T cells.

Conclusion: Our results suggest that the balance between anti-apoptotic and pro-apoptotic proteins is deregulated in $CD4^+CD28^{null}$ T cells from patients with myocardial infarction. These findings could open the way for novel therapies aimed at targeted induction of apoptosis in $CD4^+CD28^{null}$ T cells to stabilise atherosclerotic lesions.

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Interaction between glycans and the immune system: do glycans play a role in Crohn's disease pathogenesis?

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Introduction: Crohn's disease (CD) is characterized by loss of tolerance towards intestinal microorganisms, reflected by serologic responses towards fungal characteristic glycans such as mannan (anti *Saccharomyces cereviciae* antibodies), and laminarin (anti laminaribioside carbohydrate antibodies). However, the role of glycans in CD immunopathogenesis is yet unclear.

Aim: To explore glycan-induced immune responses and their correlation with intestinal inflammation.

Methods: Peripheral blood mononuclear cells (PBMCs) isolated from CD and normal control patients were stimulated by glycans or heat killed (HK) yeasts. Glycan receptor expression, cytokine secretion, and signaling pathways were assessed.

Results: The glycans mannan and laminarin induced significantly higher pro-inflammatory cytokine secretion by CD versus normal PBMCs: TNF-α (laminarin: 408 versus 212 pg/ml $P = 0.014$); IL-1β (laminarin: 284 versus 56 pg/ml, $P = 0.013$, mannan: 701 versus 279 pg/ml $P = 0.025$), IL-6 (laminarin: 2903 versus 1075 pg/ml, $P = 0.007$, mannan: 5645 versus 2856 pg/ml, $P = 0.021$). A 89% and 77% inhibition of glycan-induced TNF-α secretion was observed using syk inhibitor.

HK *C. albicans* induced higher TNF-α secretion by CD versus normal PBMCs (5876 versus 2470 pg/ml, $P = 0.05$), while HK *S. cerevisiae* induced lower IL-10 secretion by CD compared to normal PBMCs (56 versus 253 pg/ml, $P = 0.007$).

The glycan receptors: dectin-1 and mannose receptor were expressed by 60 ± 20% and 80 ± 9% of normal blood monocytes (CD14+ cells) respectively.

Conclusion: Glycans are capable of stimulating peripheral immune responses, in a Syk-dependent way. CD is characterized by hyperresponsiveness towards yeast characterizing glycans, reflected by increased pro-inflammatory cytokine secretion. This may suggest a mechanism to the existence of anti-glycan antibodies in CD patients.

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Immune dysregulation and defects in mucosal B cell homeostasis in patients with *PTEN* hamartoma tumor syndrome

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The tumor suppressor phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is the central regulator of the PI3K/AKT signaling pathway. Mice with defects in this pathway develop multiple alterations in T and B lymphocyte homeostasis leading to lymphoid hyperplasia, autoimmunity and lymphomas. The immunological consequences of PTEN deficiency in humans have not been systematically analyzed. In a series of 34 patients with heterozygous *PTEN* germline mutations we describe immune dysregulation including autoimmune lymphocytic thyroiditis, extensive hyperplastic tonsils, thymus hyperplasia and gastrointestinal lymphoid hyperplasia. Functional analysis revealed increased mTOR signaling including S6 phosphorylation within CD20+CD10+ germinal center B cells resulting in increased proliferation. Furthermore, we found reduced apoptosis of germinal center cells. By contrast proliferation in T cell areas *in situ* was normal. Part of the changes depend on the mTOR signalling pathway since it can be reversed by rapamycin treatment. Our data reveal a marked functional impact of PTEN on B cell homeostasis by modulating the PI3K/AKT signaling pathway via mTOR and anti-apoptotic signals leading to autoimmunity and lymphoid hyperplasia.

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Characterisation of EYFP expression induced by type 1 IFN production in a Trex1$^{-/-}$ RosaEYFP MxCre mouse model

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease involving autoantibodies directed against nuclear, cell surface and cytoplasmic antigens. Mutations in the *Trex1* gene has been associated with SLE, characterised by production of IFN. Trex1 is a 3′-5′ exonuclease that degrades both single stranded and double stranded DNA. Accumulation of DNA may activate the immune system resulting in a breakdown of tolerance. We have generated a Trex1$^{-/-}$ Rosa-EYFP Cre mouse line under the control of Mx1 promoter. High levels of IFN will induce Cre recombinase resulting in the removal of a stop codon in the RosaEYFP gene, causing expression of EYFP.

To determine which tissues and cells respond to *in-vivo* IFN, Trex1$^{-/-}$ Rosa-EYFP$^{+/-}$ MxCre$^{+/-}$ mice were analysed in comparison to the controls Trex$^{+/+}$ RosaEYFP$^{-/-}$ Mx-Cre$^{-/-}$ and Trex$^{+/-}$ Rosa-EYFP$^{+/-}$ Mx-Cre$^{+/-}$. Leukocytes from the blood, spleen, peripheral lymph nodes and bone marrow were analysed for EYFP and CD19, CD4, CD8 and F4/80 expression by flow cytometry. It was shown that all tissue and cell types analysed expressed EYFP, however the majority of EYFP^{+} cells were CD19^{+}. Little to no EYFP expression was observed in the control mice. Furthermore, tissue will be analysed to determine the presence of EYFP^{+} cells.

These preliminary results demonstrate that the absence of the *Trex1* gene results in the expression of IFN causing an activation of the EYFP gene. This mouse model can further our understanding of SLE by more in depth analysis of tissues types involved, the progression of the disease and the use of therapeutics.

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Candida albicans induces the expansion of regulatory helper T cells in healthy humans

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The clinical spectrum of *Candida albicans* infection ranges from harmless colonisation in most individuals through mucocutaneous disease to systemic infection. An important observation is that disease often originates from pre-existing colonisation rather than *de novo* infection. Both innate and adaptive immunity are recognised to be important in effective control of *C. albicans*. The helper T cell (Th) response to *C. albicans* is characterised by the generation of both Th1 and Th17 cells, which may be protective or, if excessive, can cause immune pathology and facilitate the spread of infection. Regulatory T cells (Treg) can inhibit Th1 or Th17 responses in other diseases, but their role in *C. albicans* infection is not known. The aim of this work was to identify and correlate the activity of circulating Th1, Th17 and Treg cells in response to *C. albicans* in healthy donors and patients with superficial *C. albicans* infection. As expected, we found that *C. albicans* antigen preparations induced both Th1 and Th17 responses in peripheral blood mononuclear cells from healthy donors and *C. albicans*-infected patients. Interestingly, Treg responses to *C. albicans* antigens were only found in healthy donors and were characterised by the expansion of nTreg and IL-10 secreting Th cells. These results raise the possibilities that the absence of Treg responses contributes to the development of infection in patients, or, conversely, the presence of Treg in healthy donors promotes commensal colonisation. In either case, manipulating Treg activity may provide a target for future clinical therapies.

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IL-10 producing B cells suppress anti-viral CD8 T cell responses in chronic HBV infection

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B cells modulate immune responses in autoimmunity, infection and cancer through antibody-independent mechanisms. In humans, a subset of regulatory B cells can suppress T cell proliferation and cytokine production through release of IL-10. IL-10 is elevated in the serum of patients with chronic hepatitis B virus infection (CHB), and is a potential candidate for suppression of HBV-specific CD8 T cell responses. We studied the contribution of IL-10 to the pathogenesis of CHB, and determined whether IL-10 producing B cells suppress T cell function in this setting.

Serum IL-10 levels were studied longitudinally in 11 patients with CHB undergoing spontaneous flares of liver disease associated with rapid changes in viral load and liver inflammation. In all individuals, IL-10 levels correlated temporally with peaks in viral load or liver inflammation. Blockade of IL-10 *in vitro* increased the frequency of HBV-specific CD8 T cell responses suggesting that these cells may be constitutively suppressed.

To investigate the role of B cells, peripheral blood leucocytes were stimulated with CpG, a toll like receptor agonist relevant in the setting of HBV, a DNA virus. IL-10 producing B cells were enriched in patients, and their frequency correlated temporally with disease flares, both after stimulation and directly *ex vivo*. Phenotypically, these cells were predominantly immature (CD19+CD24^{hi}CD38^{hi}) *ex vivo*; sorted CD19+CD24^{hi}CD38^{hi} co-cultured with peripheral blood leucocytes stimulated with HBV peptides suppressed HBV-specific CD8 T cell responses, which was partially reversed by IL-10 blockade. These data reveal a novel role for B cells in CHB.

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Interleukin-27 receptor-deficient mice develop exacerbated inflammatory arthritis associated with heightened T- and B-cell responses

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Cytokine control of adaptive immunity is a central process in the development of inflammatory diseases. T helper cells that produce interleukin-17 (IL-17; Th17 cells) are a distinct T-cell subset implicated in autoimmune diseases including rheumatoid arthritis (RA) and targeting of pathways that promote Th17 responses are currently of interest for developing alternative therapies. IL-6 family cytokines are key regulators of Th17 cells, and through differential activation of signalling transducers and activators of transcription (STAT)1 and STAT3, IL-6 and IL-27 have opposing outcomes on Th17 cell development. IL-27 is an inhibitor of Th17 cells and studies in IL-27R$^{-/-}$ mice have highlighted an anti-inflammatory role for this cytokine. However, the mechanisms linking IL-27 to arthritis progression remain unclear. To investigate this, inflammatory arthritis was induced in wild type (WT) and IL-27R$^{-/-}$ mice and disease severity assessed through measurement of joint swelling, histology and X-ray. IL-27R$^{-/-}$ mice developed exacerbated inflammatory arthritis, displaying increased synovial infiltrates and bone erosions together with heightened Th17 responses compared to WT mice. IL-27R$^{-/-}$ mice also developed increased B-cell responses associated with elevated antigen specific IgG levels. Immunohistochemistry revealed that disease exacerbation was associated with increased local activation of STAT3, further confirming a damaging role for STAT3 in the synovium. Exacerbation of inflammatory arthritis in IL-27R$^{-/-}$ mice highlights an anti-inflammatory role for this cytokine. Excessive activation of STAT3 within the joint contributes to inflammation-induced joint pathology and modulating the STAT1/3 axis may provide a potential therapy. In this regard, targeting the IL-27 signalling pathway is currently being explored.

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The regulation of arthritic bone erosions by IL-10

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Interleukin 10 (IL-10) is an immuno-regulatory cytokine that terminates the inflammatory response. In inflammatory arthritis IL-10 is elevated in the serum and synovial fluid of patients with rheumatoid arthritis (RA) and has been implicated in various pro- or anti-inflammatory processes. In IL-10KO mice, the inflammatory histopathology associated with the induction of antigen-induced arthritis was significantly enhanced and prolonged as compared to wild type (WT) controls. Interestingly, histological and radiographic analysis of joint sections from these studies suggested that IL-10 is required to prevent excessive bone degradation. Quantitative evaluation of pro-inflammatory regulators during experimental arthritis in IL-10KO mice showed specific alterations in components of the inflammasome (e.g. NALP3, caspase-1), which was accompanied by synovial increases in IL-1β expression. *Ex vivo* studies with immortalized monocytic cells showed that IL-10 could inhibit regulation of the inflammasome triggered with either alum or ATP, and suppressed production of IL-1β. In contrast, TNFα regulation (e.g. TNFα, ADAM-17) following arthritis induction in IL-10KO mice was comparable to that seen in WT. In this regard, arthritic IL-10KO mice showed similar levels of matrix metalloproteinase (MMP) activity, as assessed by *in vivo* whole body imaging and synovial MMP expression (MMP-1, MMP-3, MMP-9, MMP-13), to that observed in WT controls. These data point towards a hitherto unidentified crosstalk between IL-10 and the inflammasome, which may impact arthritic processes such as leukocyte infiltration and bone pathology.

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Genome-wide DNA methylation profiles of CD4+ CD28− T cells and its implication in the immune response

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The CD4+ CD28− T cells are a population of lymphocytes rarely found in healthy individuals, but they increase with age, inflammatory disorders, chronic infections and transplantation. Accumulation of CD4+ CD28− T cells had been associated with a reduced overall immune response to pathogens and vaccines in the elderly, and amplification of the inflammatory process in other pathologies.

In this study, we compared the global gene expression profiles of CD4+ CD28− T versus CD4+ CD28+ T subsets using Human HT-12 v3 whole genome expression arrays, and analyzed the methylation profiles with Illumina Infinium HumanMethylation27 bead chip technology. Functional analyses using Gene Ontology annotations were conducted in order to identify molecular pathways affected in the CD4$^+$ CD28$^-$ T cells. We identified a defined set of genes (559 genes) upregulated in CD4$^+$ CD28$^-$ T cells which were involved in a broad array of biological process, such as immune response, defense response, response to biotic stimulus, chemotaxis and secretion. Furthermore, 294 genes were regulated by DNA methylation in these subsets, showing hypomethylation in most of the genes associated with immunological pathways in the CD4+ CD28− T subset. Our results showed that during the differentiation to CD4+ CD28− T cells, DNA demethylation is the responsible mechanisms of a increased overexpression of genes, which contribute to the pathological functions of this subset.

Elucidation of the molecular mechanisms of this subset is important to develop strategies that can restore the changes in CD4+ CD28− T-cell functions and avoid its adverse consequences in several pathologies.

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The KIRs of HLA-B27: the role of HLA-B27 KIR interactions in infection and spondyloarthritis

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HLA-B27 (B27) is associated with resistance to infection and spondyloarthritis (SpA). We have shown that B27 can be expressed at the cell surface of antigen presenting cells (APC) as a disulphide-bonded heavy chain homodimer (termed $B27_2$) in addition to classical β2m-associated B27. $B27_2$ but not classical B27 binds to KIR3DL2 which has previously been shown to bind to HLA-A3 and A11 which are not associated with spondyloarthritis (SpA). Ligation of KIR3DL2 by $B27_2$ promotes the survival of KIR3DL2-expressing leukocytes. Here we show that $B27_2$ is a stronger ligand for KIR3DL2 than HLA-A3 and other HLA-class I. $B27_2$ dimer expressing APCs stimulated production of IL-2 by KIR3DL2CD3ε-transduced reporter Jurkat T cells to a greater extent than stimulation with APC expressing control HLA-class 1 (221B27, 813 ± 55 pg/ml; 221A3, 192 ± 23 pg/ml; 221B35, 65 ± 3 pg/ml; 221, 146 ± 3.2 pg/ml). IL-2 production was inhibited by $B27_2$ reactive and KIR3DL2-specific MAbs. KIR3DL2Fc bound $B27_2$ on transfected APCs. Ligation of KIR3DL2 by $B27_2$ stimulated IL17 production and cell survival of T cells to a greater extent than binding to HLA-A3 or other control HLA class I. Finally, peripheral blood KIR3DL2 CD4 T cells from B27 + AS patients proliferated more in response to antigen presented by syngeneic APC than T cells from healthy and disease controls. T cell survival and cytokine production was inhibited by $B27_2$ reactive and KIR3DL2-specific MAbs. Our results suggest that expansion of KIR3DL2-expressing leukocytes in B27+ SpA and B27+ healthy controls can be explained by the stronger interaction of $B27_2$ with this receptor compared to other HLA class I ligands.

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Seroepidemiology of the recent mumps virus outbreaks in Ireland

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Two recent mumps outbreaks have occurred in Ireland in 2004/2005 and 2008/2009. The aims of the study were to retrospectively investigate any potential shifts in the gender bias and age profile and to identify cohorts who are maintaining mumps virus in circulation. Two thousand six hundred cases of acute mumps infection, as determined by the presence of mumps-specific IgM in sera and oral fluids were confirmed at the National Virus Reference Laboratory. Acute mumps infection occurred more frequently in males with a ratio of approximately 2:1 in the 1−9 and 10−19 year old age groups. A 3:2 ratio was observed in the 20−29 year old cohort and the 30+ age group did not show a gender bias. Serological evidence of prior immunological exposure to mumps virus, as determined by the presence of mumps-specific IgG, was high and similar in males and females of all age cohorts (93.1−100%). A significant increase in the number of acute mumps cases in the 30 year old age group was observed. This increase was most striking in the periods between the outbreaks (71.1% in 2007 and 56.2% in 2010). In conclusion, acute mumps infection showed a male gender bias. The consistent and significant increase of mumps infection in the 30 year old age group which is also evident in the periods between outbreaks suggests that this may be the cohort maintaining the mumps virus in circulation.

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Does the build up of molecular garbage lead to inflammatory arthritis?

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The Human Leucocyte Antigen (HLA)-B27 class I molecule is key to the development of a group of inflammatory arthritic diseases. HLA-B27 is predisposed to forming heavy chain aggregates, which may participate in disease pathogenesis by inducing endoplasmic reticulum (ER) stress responses. Here we demonstrate that ER stress leads to HLA-B27 aggregate clearance by inducing ER associated degradation (ERAD) which is dependent on the ER resident chaperone calnexin and the transcription factor X-box binding protein-1 (XBP-1). Aggregate degradation requires the E3 ubiquitin ligase 3-hydroxyl-3-methylglutaryl reductase degradation (HRD)-1 and a direct interaction with ER degradation-enhancing a-mannosidase-like protein (EDEM). The data reveals the HLA-B27 dimer degradative pathway as a novel therapeutic target in the study of HLA-B27 associated arthritidies.

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Translating to the clinic biomarkers of tolerance in renal allograft recipients

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Background: Renal transplantation success is limited by the adverse side-effects associated with immunosuppressive regimens and poor long-term graft survival. The search for a safe strategy to minimise immunosuppression has led to the identification of unique biomarkers of tolerance, using high-end flow cytometry immune monitoring based at our Biomedical Research Centre (Sagoo *et al.* JCI 2010).
Aims: Our study aims to translate these defined biomarkers of tolerance into clinically useful identities, studying a large patient cohort ($n = 620$). We also propose to assess if tolerant recipients have a particular expansion of NK cell subsets.
Methods: Comprehensive flow cytometry of T cell and NK cell subsets, and RT-PCR of tolerance-related genes were performed in five groups of kidney transplants: tolerants; drug-free functionally stable, stables; functionally stable on immunosuppression, chronic rejectors; presenting signs of immunological rejection and healthy controls.
Results: Preliminary flow cytometry and RT-PCR biomarker data is consistent with our ongoing validation. In addition, chronic rejectors represented the highest percentages of $CD56^+CD3^-$ NK cells and the cytotoxic subset ($CD56^+NKG2D^{Hi}$) whereas the tolerant group represented a regulatory profile ($CD56^+CD25^+HLA\text{-}DR^{Lo}$). Tolerants also showed a decreased percentage of proinflammatory $CD14^+CD16^+$ monocytes.
Conclusions: An effector and activated NK phenotype dominated in chronic rejectors, compared to tolerants who demonstrated a regulatory NK cell phenotype. Tolerants also showed a less potent proinflammatory blood monocyte phenotype. This ongoing data will be used to validate the set of biomarkers of tolerance for a clinically applicable test and help unravel the mechanistic role of these immune cells in transplant tolerance.

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Epstein–Barr virus gene expression, human leukocyte antigen alleles and chronic high viral loads in paediatric renal transplant patients

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Studies have identified solid organ transplant recipients who remain asymptomatic despite maintaining chronic high Epstein–Barr virus (EBV) viral loads. We examined clinical manifestations, EBV gene expression, human leukocyte antigen (HLA) alleles, and specific T-cell responses to EBV infection in pediatric renal transplant patients. Seventeen pediatric renal transplant patients were categorized according to EBV viral load into those with chronic high viral loads (CHL) and recipients who resolve EBV infection (REI). EBV gene expression was analyzed using real-time PCR assays and EBV-specific T cells were analyzed by flow cytometry. EBV gene, EBV-encoded small RNA 1, was expressed at significantly higher levels in CHL compared with EBV seropositive controls ($P < 0.005$) and raised compared with REI. BamHI A right-ward transcripts were also expressed at higher levels in CHL patients ($P < 0.03$) than in REI. Expression of latent genes, EBNA1, LMP1, LMP2, and lytic gene BZLF1 were restricted to the CHL group with viral gene expression varying over time. HLA-A*02 allele expression was predominant in CHL patients (80%) and GLClytic-specific cytotoxic T-lymphocytes were absent. In contrast, HLA-B*08 allele expression was prevalent in REI patients (71%) and RAK lytic cytotoxic T-lymphocytes were detected in all patients. EBV gene expression in CHL carriers differs from those that resolve infection and should be interpreted alongside HLA polymorphisms.

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Head and neck cancer patients with HPV-associated tumours exhibit a different profile of costimulatory receptors on $CD4^+$ T cells and of circulating angiogenic factors

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Background and aim: Head and neck cancers (HNC) are aggressive tumours that associate with an inability of T cells to control tumour progression. Expression of Human Papilloma Virus (HPV)-derived protein p16 in HNC is known to correlate with a better prognosis than p16-negative tumours. Our aim was to dissect the immune mechanisms that may explain this phenomenon.
Methods: The expression of costimulatory (ICOS, OX40, 4-1BB) and coinhibitory (CTLA-4, PD-1) receptors by $CD4^+$ T cells from 18 newly diagnosed HNC patients and controls was analysed by flow cytometry. In addition, a panel of 11 pro- and anti-angiogenic factors was analysed in serum samples of HNC patients and controls using multiplex ELISA. Levels of costimulatory receptors and angiogenic factors were analysed with respect to the p16 expression in the tumoral tissue.
Results: We found that costimulatory receptors OX40 and 4-1BB (which control the function of T cells) were expressed at significantly lower levels on $CD4^+$ T cells from HNC patients compared to healthy controls. No differences were observed in the levels of coinhibitory receptors. Of note, the co-stimulatory receptor ICOS was lower in p16-negative patients, suggesting decreased T-cell function in this patient group. Interestingly, p16-negative patients exhibited higher levels of proangiogenic factors angiopoietin, VEGF and PIGF compared to p16-positive HNC patients.
Conclusions: HNC patients that lack p16 expression have a different pattern of costimulatory receptors and angiogenic factors that seems to confer a more aggressive tumour phenotype. Better understanding of these changes may guide the development of novel therapeutic targets.

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Peripheral blood mononuclear cells (PBMCs) and adipose tissue from overweight and obese individuals express significant high levels of matrix metallopeptidase 9 (MMP-9)

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Obesity is a growing epidemic and is associated with metabolic diseases such as insulin resistance, hypertension, and cardiovascular disease. Chronic inflammation is one of the major consequences, which results in dysfunction of adipose tissue in obese patients. Growing evidence suggests a role for MMP-9 as a key component in migration of monocytes/macrophages to adipose tissue. Several studies have showed increased levels of plasma/serum MMP-9 obtained from obese individuals; however the source of MMP-9 production has not been investigated. Therefore, we aimed to identify the sources of MMP-9 production in obese individuals. Plasma concentrations of MMP-9 were measured in samples isolated from 76 individuals by ELISA assay. mRNA expression of MMP-9 in PBMCs was quantified by RT-PCR analysis. MMP-9 expression was also determined in the adipose tissues of these subjects using immunohistochemistry analysis. Overweight and obese individuals showed a significant increase of MMP-9 expression in both PBMCs and adipose tissue when compared with lean individuals ($P < 0.05$). Interestingly, a remarkable higher expression of MMP-9 was observed in overweight and obese individuals with type II diabetes ($P < 0.05$). These findings indicate that the PBMCs are the more prone to release and produce MMP-9 in obese individuals. Further investigations are being carried out to determine the cellular compartments within the PBMCs and/or adipose tissue that are directly involved in the modulation and induction of MMP-9 in obese and diabetic individuals.

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Genotype-dependent expression of the transmembrane isoform of CTLA-4 in FOXP3$^+$ Tregs

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CTLA-4, an inducible negative regulator of T cell activation, plays a critical role in the maintenance of immune self tolerance and homeostasis. Contrary to conventional T cells, CTLA-4 is constitutively expressed by thymus-derived FOXP3$^+$ regulatory T cells (Treg) and is essential for their function. Genetic variation of *CTLA4* has been associated with susceptibility to several human autoimmune diseases including type 1 diabetes (T1D). Two alternatively spliced CTLA-4 mRNAs are expressed in human T cells, one encoding the transmembrane molecule (TM CTLA-4) and another that lacks the transmembrane domain that has been described previously as soluble CTLA-4 (sCTLA-4). We and others found significantly lower levels of sCTLA-4 mRNA in purified CD4$^+$ T cells and FOXP3$^+$ Tregs from donors carrying the *CTLA4* SNP CT60 (rs3087243) T1D susceptible genotype as compared with donors having the protective genotype. Single-cell polychromatic flow cytometry analysis of CD4$^+$ T cells from 17 pairs of age-matched donors differing by genotype at CT60 demonstrated that intracellular CTLA-4 protein levels in Tregs are significantly decreased in donors carrying the T1D susceptible *CTLA4* genotype both *ex vivo* and after short-term activation. Using novel polyclonal and monoclonal antibodies that are specific for sCTLA-4's unique C terminal amino acids, we have determined that CD4$^+$ T cells and Tregs produce little or no sCTLA-4 protein. Therefore, TM CTLA-4 protein is responsible for the genotype-dependent CTLA-4 expression difference that we have observed in FOXP3$^+$ Tregs. These results suggest that an inherited decrease of Treg function predisposes to T1D and other autoimmune diseases.

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Candida albicans promotes the differentiation of induced CD4$^+$CD25$^+$Foxp3$^+$ regulatory T cells

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Candida albicans is commonly found in the microbial flora of humans and although this commensal relationship is usually stable, more serious infection can occur, ranging from superficial to disseminated disease. Regulatory T cells (Treg) have an important role in limiting helper T cell (Th) responses and maintaining immune homeostasis during infection. Foxp3$^+$ Treg are divided into subsets based upon expression of the transcription factor Helios, which has been described as a marker of thymically-derived natural Treg (nTreg), and not induced Treg (iTreg). We demonstrate, for the first time, that *C. albicans* promotes Th cell differentiation towards an iTreg phenotype and that these cells are less able to suppress *C. albicans*-induced Th responses compared to nTreg. Splenocytes isolated from a disseminated *C. albicans* mouse infection were exposed to *C. albicans* antigens *in vitro*. An increase in the number of CD4$^+$CD25$^+$Foxp3$^+$ cells was observed in *C. albicans*-stimulated splenocyte cultures from infected, but not control mice. Interestingly, these Treg adopted an iTreg phenotype, whereas Treg from control mice remained predominantly nTreg, as measured by Helios expression. Importantly, CD4$^+$CD25$^+$ Treg isolated from *C. albicans*-infected mice were less able to suppress Th proliferative responses to *C. albicans* antigens, compared to those from controls. These data suggest that *C. albicans* induces the differentiation of iTreg during infection, but that these cells may be weak suppressors, potentially allowing the expansion of effector Th responses. Further work will investigate whether nTreg or iTreg suppress particular Th subset responses and if this alters disease progression.

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Familial recurrent herpes-simplex type 1 infection: an approach to characterise the molecular basis

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In most people, primary infection with the herpes-simplex type 1 virus (HSV-1) occurs in childhood and is followed by latent infection. While many individuals may experience occasional re-activation typically manifesting as cold sores, gingiovostomatitis or skin lesions in the perioral area, a minority exhibit more frequent recurrences. It is estimated that 80–90% of the adult population are seropositive to HSV-1 and have therefore had a primary infection. In the general UK adult population, 42% complain of recurrent HSV with the majority having <4 episodes per year. However, approximately 1% have more than seven attacks per year. It is well recognised that frequent reactivation is associated with certain immunodeficiency states, e.g. acquired immunodeficiency syndrome (AIDs) and therapeutic immunosupression. However, in the absence of these conditions it is not well understood why some individuals are particularly susceptible to recurrences. Although the virulence of the infecting strains is thought to play a role, genetic variation that influences the host immune response is also likely to be important.

A patient who was reported to be having frequent recurrences of HSV-1 (>6 per year) despite acyclovir prophylaxis was referred to our clinic in order to investigate whether she was immunodeficient. She came from an unusually large family and reported that several family members across three generations had symptoms consistent with recurrent HSV. We will describe the results of the immune investigations of the index patient and our strategy to characterise the disease in the wider family and investigate its molecular basis.

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Early expansion of T-cells over 14 days post stem cell transplant predisposes patients to acute graft versus host disease

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Stem cell transplant (SCT) is an accepted strategy for the treatment of high risk haematological disease and involves replacement of the recipient's immune system with that of an HLA matched individual. T-cell reconstitution post-SCT is critical in induction of both the graft versus leukaemia effect (GVL) and graft versus host disease (GVHD), and thus in determining the outcome of SCT. However, as thymic output is limited in adult leukaemia patients, thymic-independent pathways, primarily homeostatic proliferation, will be key to the shaping of the adaptive immune response.

Here we characterised the T-cell compartment of patients in the first 14 days post-SCT. Strikingly, during the second week post transplant, both CD4 and CD8 T cells numbers were increased two and fourfold respectively in a cohort of patients who then went on to develop GvHD ($n = 3$). In contrast, numbers remained static in patients who did not develop disease ($n = 3$). The increase in cell numbers in GvHD patients was associated with (i) an increase in lymphocyte forward light scatter, indicating the presence of T-cell proliferation (ii) expansion of both naive and effector memory populations, and (iii) an increased potential to secrete IFNg and IL-17 production, with reduced IL10 production. Thus, early expansion of mature T cell populations with a pro-inflammatory phenotype post transplant may predispose patients to GVHD.

To our knowledge, this is the first work characterising T-cell reconstitution in the first 14 days post-transplant and highlights the previously over-looked importance of lymphocyte activity in determining SCT outcome during this time.

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Protection at mucosal barriers: the effect of multiple-challenge infections on the susceptibility of Muc5ac mice to *Trichuris muris*

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We have recently demonstrated that expression of the mucin Muc5ac, normally expressed in non-intestinal mucosa, is highly up-regulated in the intestine following infection with the caecal-dwelling nematode *Trichuris muris*. Moreover, genetic deletion of Muc5ac leads to complete susceptibility to a high dose infection in an otherwise resistant mouse strain, indicating Muc5ac is a major effector mechanism involved in protection against this parasite. It has also been determined that the expression of Muc5ac is IL-13-dependent since up-regulation of this mucin does not occur in *T. muris* infected IL-4Rα mice. In nature, multiple-challenge infections represent the typical manner in which host-acquired immunity is generated. Muc5ac null mice were infected repeatedly with small numbers of *T. muris* eggs to mimic a more natural infection. Accumulative dosing in this manner caused the generation of resistance in Wt mice which correlated with increased expression of IL-13. Work presented here demonstrates how Muc5ac expression levels changed following multiple-challenge infections and utilising Muc5ac null mice, the role of this mucin in this infection scenario was explored. Moreover the immune response that developed in the Muc5ac mice was investigated over the course of infection, with particular focus on the Th2 response and the expression of IL-13.

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Phenotypic analysis of colonic macrophages in CX3CR1$^{+/eGFP}$ mice infected with the parasitic nematode *Trichuris muris*

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Trichuris muris is a nematode parasite of the mouse which dwells in the large intestine. It is a natural mouse model of *Trichuris trichiura*, one of the most prevalent human helminth parasites worldwide. While the generation of a Th2 response plays an essential role in a successful immune response, the local effector mechanisms which bring about worm expulsion from the gut, and the regulation of the inflammatory response are not yet fully understood. Previous studies in this laboratory have shown that macrophages accumulate in the large intestine post-infection and are the predominant type of inflammatory cell. This study aims to determine the phenotype of these cells. Leukocytes were isolated, by enzymatic digestion, from the large intestine of CX3CR1$^{+/eGFP}$ mice and the macrophages therein were analysed by multi-colour flow cytometry. In this mouse model, cells expressing the chemokine receptor CX3CR1 also express eGFP. Macrophages were defined as F4/80^{+}CD11b^{+}I-A/I-E^{+}Siglec-F^{-}. Two contrasting populations of CX3CR1^{+} macrophages were identified. The first, F4/80^{high}-CX3CR1^{high} and largely Ly6C^{-}CCR2^{-}TLR-2^{-}, was relatively abundant in uninfected mice. This phenotype is consistent with resident macrophages. In contrast, the second, F4/80^{low}CX3CR1^{low}, was relatively abundant post-infection. In both naïve and infected mice, a high proportion of these macrophages were Ly6C^{+}CCR2^{+} whereas TLR-2 was only expressed post-infection. This population is consistent with inflammatory macrophages. Our data describe, for the first time, the changes which occur to resident and inflammatory macrophages following infection with a gut-dwelling helminth.

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The early-life gut microbiota associates with cytokine production at 2 years of age

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Background: Microbial exposure early in life influences immune maturation and potentially also the development of immune-mediated disease.
Objective: To study early-life gut colonization, in relation to cytokine responses at birth and at 24 months.
Methods: DNA was extracted from fecal samples at four time-points (1 week–2 months) and Real time PCR was used for detection of *Bifidobacterium (B.) adolescentis*, *B. breve*, *B. bifidum*, a group of Lactobacilli [*Lactobacillus (L.) casei*, *L. paracasei* and *L. rhamnosus*] as well as *Staphylococcus (S.) aureus*. Mononuclear cells from peripheral blood (PBMCs) at 2 years of age, were stimulated with PHA and numbers of IL-4, IL-10, IL-12 and IFN-g secreting cells were evaluated using ELISpot. Further, PBMCs were stimulated *in vitro* with *S. aureus* or Lactobacilli.
Results: Infants persistently colonized with Lactobacilli had fewer IL-4 ($P = 0.030$) and IL-12 ($P = 0.022$) producing cells at age two following PHA stimulation. In contrast, early *S. aureus* colonization associated with higher numbers of IL-4 ($P = 0.022$), IL-10 ($P = 0.016$), IL-12 ($P = 0.019$) producing cells. Early co-colonization with Lactobacilli and *S. aureus* associated with suppression of IL-4 ($P = 0.004$), IL-10 ($P = 0.004$), IL-12 ($P = 0.003$) and IFN-g ($P = 0.034$) secreting cells compared to colonization with *S. aureus* alone. Also, *in vitro*, *S. aureus* induced a strong cytokine response by PBMCs, which was dampened by the simultaneous presence of Lactobacilli.
Conclusion: We demonstrate that the early colonization pattern associates with the PHA-induced cytokine profile at 2 years of age. Dysbiosis in the early microbiota may modulate the risk of developing inflammatory conditions like allergy.

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Development of a virus immunocapture method to determine cellular sources of replicating populations of HIV-1 *in vivo*

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Although CD4 lymphocytes represent the main cellular target for HIV-1, it is increasingly recognised that CD8 lymphocytes can also be infected both *in vitro* and *in vivo* after activation. Increasing infection frequencies during disease progression suggest targeting of activated CD8+ cytotoxic T-lymphocytes (CTLs) substantially contributes to immunodeficiency and represents a significant reservoir of HIV-1. HIV-1 derived from infected CTLs incorporate CD8; immunocapture demonstrated that 10–30% of plasma virus was CD8-derived (Hughes et al., 2008). Virus capture using biotinylated antibodies and MyOne Straptavidin beads (Invitrogen) allows a variety of other cellular markers to be detected. We have documented the incorporation of different cellular proteins (L-selectin, integrins $\alpha 4$, $\beta 1$, $\beta 7$, CD103) in virions on *in vitro* infection of cell lines and sorted PBMCs. Capture of CD103 has been used to explore the targeting of gut-associated lymphoid tissue during HIV-infections by comparing its incorporation in plasma-derived virions from acutely infected and asymptomatic individual (early) and those with AIDS (late disease progression). Whereas capture of most integrins was similar in early and late stages of infection, a significant increase in CD103-captured virus was consistently observed during HIV infection (0.8% versus 8.8%, $P = 0.0026$). In cell culture CD103-upregulation was largely restricted to CD8+ lymphocytes on activation. Its greater incorporation into plasma virions indicates a higher infection frequency of CD8+ T-cells residing in the gut in chronic infection. The use of virus immunocapture to detect incorporated cellular proteins in virion envelopes will find wide application for determining virus reservoirs and cellular targeting during progression to AIDS.

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Endogenous IL-21 regulates pathogenic CD4 T cell responses during respiratory viral infection

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Interleukin-21 (IL-21) is a cytokine of great interest because of its ability to regulate Th1, Th2, and Th17 differentiation. It is involved in the pathogenesis of several diseases including autoimmune conditions (rheumatoid arthritis), cancers (lymphomas) and viral infections (HIV) but its role in immunity to respiratory infection has not been studied. To test its effects in RSV disease with a Th2 profile, BALB/c mice were vaccinated with recombinant vaccinia virus expressing the RSV G protein. On RSV challenge, immunised mice developed augmented disease characterised by enhanced CD4 T cell recruitment, activation and Th2 cytokine production peaking on days 4–5 post RSV challenge. IL-21 depletion at vaccination caused subsequent RSV clearance to be reduced and virus-specific serum antibody responses to be impaired. However, RSV challenge caused exacerbated pathology with enhanced lymphocyte, neutrophil, and APC recruitment and increased bronchoalveolar lavage IFN-γ and IL-17 levels. Lung CD4 T cells producing IFN-γ and IL-17 were more numerous in depleted mice and CD4 T cells from depleted mice expressed more *rorct* mRNA post priming, and increased *rorct*, and *tbx21* mRNA post RSV challenge indicating enhanced Th1 and Th17 cell differentiation. Furthermore, adoptive transfer of splenic CD4 T cells from primed, IL-21-depleted mice into naïve recipients replicated many of the effects previously observed after RSV infection, indicating that IL-21 mediates its effects via CD4 T cells in this model. IL-21 is therefore key modulator of antiviral immunity in the lung, regulating T and B cell responses and having potent and specific effects on viral lung disease.

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Airway epithelial cell inflammatory responses are increased by IL-17 in the presence of RSV infection

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Introduction: Respiratory syncytial virus (RSV) is a major cause of bronchiolitis in infants. It preferentially infects and replicates in the respiratory epithelium causing airway inflammation. In RSV-infected airways, elevated interleukin (IL)-17 levels have been found which correlate with airway responsiveness. Interestingly, IL-17 enhances the inflammatory effects of other cytokines such as TNF-a and IL-1b. The aim of this study was to examine the inflammatory effects of IL-17, IL-13 (a Th2 cytokine associated with airway hyper-responiveness) and IL-17 + IL-13 together on airway epithelial cells, in the context of RSV infection.

Methods: Immortalised bronchial epithelial cell cultures (BEAS-2B) ($n = 3$) were stimulated with 1, 10 and 100 ng/ml of IL-17 with and without RSVA2 (0.25MOI) for 24 and 48 h. Similar treatments with IL-13 and IL-17 + IL-13 were also performed. Culture supernatants were collected for IL-6 and IL-8 analysis by ELISA.

Results: Both IL-6 and IL-8 production were significantly raised in RSV-infected cultures treated with IL-17 compared to non-infected cultures. The same effect was not observed with IL-13 and no synergistic effect was seen with IL-17 + IL-13.

Conclusions: In the presence of RSV infection, IL-17 significantly increases pro-inflammatory cytokine expression in airway epithelial cells. No synergistic effect was observed with IL-17 and IL-13.

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Endogenous soluble CTLA-4 as a regulator of autoreactive pathogenic immune responses in systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease causing widespread, serious damage to many organs, including the joints, kidney and brain. The disease arises from dysregulated T and B cell responses resulting in production of autoantibodies against nucleosomal and spliceosomal antigens. Increased bioavailability of type 1 interferons may also contribute to breakdown of self-tolerance, and high serum levels of IFN-α correlate with disease activity in SLE patients. A single nucleotide polymorphism associated with lupus susceptibility, called CT60, appears to regulate expression levels of an alternatively spliced soluble CTLA-4 (sCTLA-4) transcript, but any relationships between sCTLA-4, IFN-α and SLE remain to be established. In this study we investigate the functional role of sCTLA-4 in controlling immune responses to a panel of nucleosomal peptide autoantigens by cultures of peripheral blood mononuclear cells (PBMC) from panels of SLE patients and healthy donors. There was no significant increase in cell culture supernatant levels of sCTLA-4 in lupus patient responses to any autoantigenic peptide at a range of concentrations. However, basal levels of sCTLA-4 in culture supernatants, correlated significantly with culture supernatant levels of IFN-α, indicating that sCTLA-4 may also be a useful marker of disease activity. Furthermore, our data raise the possibility that sCTLA-4 plays an innate role in regulating IFN-α and the autoimmune responses in SLE.

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High frequencies of $CD8^+CD57^+$ T-cells are correlated with CMV-serostatus

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Aim: To determine whether the frequencies of $CD8^+CD57^+$ T cells can reliably indicate CMV seropositivity. We compared CMV specific IgG titres, T cell responses to CMV peptide-antigens and the percentage of CD57-expressing CD8 T cells.

Method: CMV serology (IgG) was performed on serum samples from CMV seropositive and negative, young (20–30 years) and older (>60 years) healthy donors. Multi-colour flow cytometry was used to characterise CD57-expression on CD8 T-cells using heparinised whole blood. T-cell reactivity against 20 different protein-spanning peptide pools was determined on PBMC using an intracellular staining (ICS) assay using the activation markers CD40L, CD107a, TNF-α, IFN-γ, IL-2.

Introduction: CD57 is a marker of terminal T-cell differentiation. Several studies have observed an increase in $CD8^+CD57^+$ T cells in individuals with chronic conditions (with immune deficiency component) such as, CMV infection, autoimmune diseases and in transplant recipients.

Result: ROC curve analysis identified optimum cut-offs for the percentage of $CD57^+$ CD8 T-cells that were different in the young (12%) and the older individuals (23%). Several CMV seronegative (IgG) individuals exhibited CMV-specific T-cell responses and as a result ROC curve analysis was repeated with respect to T-cell responsiveness as target variable.

Conclusion: A number patients that were CMV negative according to serology show a T cell response to CMV. The frequency of $CD8^+CD57^+$ T-cells is a better indicator of CMV T cell responsiveness than IgG serology.

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Secondary progressive uveitis is characterised by a late accumulation of cytotoxic lymphocytes

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Experimental Autoimmune Uveitis (EAU) is a model for human non-infectious intraocular inflammation (uveitis). This $CD4^+$ T cell mediated disease can be induced with peptides from the retinal autoantigen retinol binding protein-3 (RBP-3; also known as interphotoreceptor retinol binding protein; IRBP). Many different leukocyte populations infiltrate the retina at different stages of disease during RBP-3 peptide induced EAU. The purpose of the study was to analyse the inflamed ocular environment during the secondary progressive phase of disease.C57BL/6 mice were immunised with RBP-3 1–20 peptide. Disease severity was assessed by Topical Endoscopic Fundal Imaging (TEFI), cellular infiltrate by flow cytometry. Cytokine production was measured by intracellular cytokine staining. EAU induced with 1–20 peptide led to a chronic and persistent expression of disease. After the primary peak, the inflamed eye has a significant increase in CD4 IL-17 producing cells compared to the spleen. Furthermore as the disease progressed, there was a late and significant increase in CD8 T cells and natural killer (NK) cells manifest between days 38 and 43. The CD8 T cell infiltrate shows little IFNγ and IL-17 cytokine production and lacks the ability to degranulate, as shown by negligible CD107a expression. In contrast, NK cells infiltrating the eye have a high expression of CD107a. In conclusion, analysis of retinal infiltrate in the secondary progressive phase of EAU reveals distinct changes in the balance of the infiltrating cell populations that may play a role in perpetuating or regulating disease.

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Identification of susceptibility loci associated with immunopathogenesis of bovine tuberculosis

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Bovine bovine tuberculosis (TB), caused by *Mycobacterium bovis*, remains a serious problem in the UK. Control involves routine skin testing with *M. bovis* antigens and the slaughter of test-positive animals. Recent evidence has shown that host genetic variation influences susceptibility to bTB, and resistance is likely to be mediated by innate and adaptive immune responses. Improved understanding of the immune mechanisms of host susceptibility/resistance has the potential to improve control of bTB. To identify loci associated with variation in TB susceptibility a case/control genome wide association study (GWAS) was performed using the newly available Illumina bovine high density SNP chip. DNA samples were collected from 464 herds of the Northern Ireland Holstein-Friesian dairy cattle population between 2008 and 2009. Cases were sampled at slaughter, and were defined as animals with both a positive reaction to the tuberculin skin test and a confirmed bTB lesion. Age matched controls were sampled from a subset of case herds, and were defined as animals that were negative to the tuberculin skin test. After QC edits, 1161 cattle (596 cases and 565 controls) and genotype data from 617 639 SNPs remained. A chi-squared allelic test was used to test for associations between individual SNPs and resistance to bovine TB. In this study, 40 loci were associated with resistance to bTB ($P < 0.00001$). Future work will involve replication of findings, and fine-mapping of GWAS regions, followed by the identification causal variants and plausible candidate genes.

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Characterizing T cell responses associated with hypersensitivity pneumonitis in pigeon fanciers

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Around 10% of pigeon fanciers are affected by a form of hypersensitivity pneumonitis known as pigeon fanciers' lung (PFL). PFL is an aberrant inflammatory immune response to dust particles that contain pigeon antigens, inhaled during pigeon husbandry, and manifests in fever and dyspnoea. A lymphocytic infiltrate has been observed in lung biopsies and brochoalveolar lavage as well as Th1 cytokines. However, little is known about the implicated pathogenic T cells and whether a systemic response can be measured that indicates disease presence or severity. Disease history and blood samples were taken from 40 pigeon fanciers at a local pigeon show, half of which had PFL. Effector T cell responses were examined by ELIspot, and all pigeon fanciers showed an IFNγ response against antigens in pigeon serum regardless of possessing PFL, and there was no difference in magnitude of response between the groups. No pigeon-specific IL-4, IL-5, IL-13 or IL-17 ELIspot responses could be detected in any person. Although a cultured ELIspot assay revealed lower memory responses than for common recall antigens, CFSE dilution demonstrated proliferation of CD4 cells in response to pigeon antigen that also possessed CD49d, a potential lung-homing integrin. Interestingly, the IFNγ ELIspot response could not be inhibited by anti-MHC class II antibody. These findings show that an unconventional T cell response is generated in conjunction with pulmonary exposure to pigeon antigens that may be necessary but not sufficient to cause PFL disease, and so further analysis of these cells is warranted.

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Characterisation of Th17, Th22 and regulatory T cells in atopic eczema

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The balance between different subsets of CD4+ T helper (Th) cells is a key determinant of the outcome in many inflammatory and infectious diseases, including eczema. The Th17 pathway has been reported to be deficient in chronic atopic dermatitis compared with psoriasis. Recent advances in knowledge of CD4+ Th subsets raise questions as to the presence and roles of related Th17, Th22 and T regulatory cell (Treg) populations in eczema.

The aim of this work was to characterise and enumerate isolated lymphocytes from atopic eczema lesions and uninvolved skin *ex vivo* using flow cytometry. Changes in these cell populations *in vivo* were also determined in samples from uninvolved skin 24, 48 and 72 h after topical application of house dust mite extract (HDM).

Our study shows that IL-17A producing CD4+ Th17 cells are recruited or expanded in the contact dermatitis response to HDM (3−12% CD4+ cells). However, in chronic lesional skin IL-22 + CD4+ T cells (5−20%) are present in equal or greater numbers than IL-17A + CD4+ T cells (0−10%) suggesting that Th22 cells form an integral part of the disease phenotype. Treg are also present in lesions (6% of CD4+ population).

These data confirm Th17 cells as prominent in eczematous skin, particularly in acute exacerbation, but can be matched by increased numbers of Th22 cells in chronic atopic dermatitis lesions. Although regulatory cells are present they are either defective, or present in insufficient numbers to control effector responses. Manipulating such a balance could be the basis for novel forms of therapy.

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Impact of nitration on the immunogenicity of Bet v 1.0101

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Nitration of Bet v 1.0101 (Bet) can occur exogenously and endogenously, and might be one explanation for the higher prevalence of allergy in industrialised countries. Previously, nitration was shown to influence processing of allergens by dendritic cells (DCs). Stimulation with nitro-Bet (Bet nitrated with tetranitromethane in MeOH) increased the number and specificity of the MHC class II presented peptides when compared to mock-Bet (MeOH only). To further study the effects of nitration, uptake experiments with labeled allergen were performed and showed a higher uptake of nitro-Bet compared to mock-Bet. In addition, IgE reactivity against freshly nitrated Bet and mock-Bet was tested in 100 patient sera by ELISA. The results showed no increased IgE response towards nitro-Bet versus mock-Bet. The biophysical changes occurring upon nitration were followed by gel electrophoresis and size exclusion chromatography, with as main result the induction of SDS resistant allergen oligomerisation of nitro-Bet. To study the effects of nitration on DC-activation, monocyte derived DCs were stimulated with Bet, mock-Bet and nitro-Bet. The analysis of secreted cytokines reveals high concentrations of the Th1 promoting cytokines IL-12, IL-6 and TNF-α upon stimulation with Bet, which is probably due to lipopolysaccharide (LPS) bound to the allergen. Notably, this Th1 priming milieu is absent in the case of nitrated Bet. This major difference between the nitrated and untreated allergen is currently investigated more detailed, in the presence and absence of LPS.

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The role of regulatory T cells in the rheumatoid arthritis joint

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Regulatory T (Treg) cells play a crucial role in maintaining tolerance and preventing autoimmunity. Treg cells suppress effector T cell proliferation and proinflammatory cytokine production, including IL-17 and IFN-γ. However it has been shown that Treg cells can exhibit functional plasticity and secrete cytokines. Despite the fact that Treg cells are present at a relatively high frequency in the rheumatoid arthritis (RA) joint, inflammation persists. We therefore examined the suppressive capacity and plasticity of Treg cells in synovial fluid (SF) from the RA joint. Using a flow cytometry based suppression assay we were able to assess the contribution of both effector cells and Treg cells to proliferation and cytokine production. High levels of IFN-γ were produced by effector cells from SF, which was effectively suppressed by the SF Treg cells as was proliferation. In contrast, the addition of SF Treg cells to SF effector cells increased the production of IL-17 by effector cells. Furthermore, in an indirect suppression assay the depletion of Treg cells from CD4 T cells resulted in substantially increased IFN-γ but decreased IL-17. IFN-γ has been shown to negatively regulate IL-17, suggesting that suppression of IFN-γ by Treg cells could be responsible for the increased IL-17 production. We also observed that a significant proportion of SF Treg cells produced IL-17 and IFN-γ. In conclusion our data suggest that IL-17 and IFN-γ may be reciprocally regulated in the RA joint and that SF Treg cells can exhibit plasticity. These mechanisms may contribute to inflammation in the joint.

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Fractalkine production mediates virulence-associated monocyte recruitment and *Mycobacterium tuberculosis* infection

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Mycobacterium tuberculosis-induced cellular aggregation is essential for granuloma formation and may assist establishment and early spread of *M. tuberculosis* infection. The *M. tuberculosis* RD1 mutant, which has a non-functional Esx1 type VII secretion system, induced significantly less production of the host macrophage-derived chemokine fractalkine (CX3CL1). Upon infection Esx1-dependent fractalkine production mediated CD11b+ monocytic cell recruitment and increased infection of neighbouring cells consistent with early local spread of infection. At disease sites in humans, fractalkine levels were associated with increased CD11b+ monocytic cellular recruitment and extent of granulomatous disease. These preliminary findings suggest a novel Esx1-mediated, fractalkine-dependent mechanism in early tuberculous disease pathogenesis in humans.

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Inducible functional tertiary lymphoid structures, autoimmunity and exocrine dysfunction in wild-type mouse salivary glands via local adenoviral delivery

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Objectives: Salivary glands of Sjögren's syndrome (SS) develop tertiary lymphoid structures (TLS) with B/T cell compartmentalization, follicular dendritic cell networks (FDCs), high endothelial venules (HEV) and expression of activation-induced cytidine deaminase (AID). The mechanisms triggering TLS formation, autoimmunity and exocrine dysfunction are largely unknown. Here we present a novel model of inducible TLS following administration of adenovirus-5 (Ad5) in the submandibular glands (SGs).
Methods: Luciferase- or LacZ-encoding Ad5 were delivered in C57BL/6 mice SGs through retrograde cannulation. SGs were analysed at 5, 12 and 18 days post-infection (dpi) for lymphoid aggregate, T/B cell segregation, FDCs, HEV and submandibular flow. Expression of TLS-related genes was investigated by TaqMan-PCR. Autoantibodies were detected by IF and ELISA.
Results: Grade-1 (<50 cells) and grade-2 (>50 cells) aggregates developed at 12 dpi showing initial T/B cell segregation and HEV. At 18 dpi fully-formed TLS with FDCs were present in 100% of the cannulated glands. At 12 dpi a peak in the expression of Ltβ (40-fold increase), CXCL13 (200-fold), CCL19 (90-fold), CXCR5 (1000-fold) and CCR7 (70-fold) was observed. TLS were functional as demonstrated by AID expression and Im-Cg circular transcripts. SG flow reduction was clearly observed at 12 dpi (approximately 45%). Finally, 70% of mice developed anti-nuclear antibodies.
Conclusions: Here we present a novel inducible model of sialoadenitis in response to Ad5 administration characterised by functional TLS, decrease in salivary flow and development of autoimmunity. This model has the potential to unravel the cellular and molecular mechanisms regulating TLS formation, exocrine dysfunction and autoimmunity in SS.

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The prodrome in experimental autoimmune uveoretinitis (EAU) is conditioned by a non-specific increase in immunosurveillance

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Experimental autoimmune uveoretinitis (EAU) is a model of posterior uveitis, an autoimmune disease of the retina, which causes severe visual impairment in over 30% of affected patients. The EAU model has a proven track record in identifying relevant clinical targets and manifests many of the pathological features of human disease. EAU is produced by immunisation with a tissue specific peptide antigen combined with complete Freund's adjuvant and pertussis toxin. Because the normal eye contains a very small number of leukocytes, we are able to quantify immunosurveillance with a high degree of sensitivity. Like many induced autoimmune models, immunisation with the target autoantigen is followed by a clinically silent phase. Using multiparameter flow cytometry and topical endoscopic fundal imaging (TEFI), we have characterised this window in terms of cellular infiltrate. Immunising with an autoantigen or with an irrelevant foreign peptide leads to the accumulation of CD4 positive T lymphocytes and Ly6G positive leukocytes that can be detected within the ocular tissue from day 5. At this time the eye is clinically normal. Comparing the CD4 cells in the disease and control groups demonstrates a larger number of cells when animals are immunised with the pathogenic peptide. We conclude that the immune system responds to non-specific signals by upregulating immunosurveillance of the immune privileged retina, and that the addition of an antigen specific signal poises the retina with a retained population of antigen specific cells, primed to initiate frank clinical disease.

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Killer-cell immunoglobulin-like receptor (KIR) genetic diversity in a unique HIV-1 cohort in China

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Background: KIR molecules are primarily expressed on Natural Killer (NK) cells. Their interactions with HLA molecules regulate NK cell activity in modulating the outcome of both infectious and auto-immune diseases. Epidemiological findings have implicated certain KIR-HLA genotypes with delay HIV-1 progression (1, 2) and susceptibility to HIV-2 infection (3). The present study examines immunogenetic factors in a unique Chinese cohort of former plasma donors that was accidentally infected with HIV-1 in the mid-1990s. When samples were first collected in 2003, approximately 8–9 years after infection, a number of people unfortunately had died from AIDS, but many of the survivors have good control over the virus to-date without the help of antiretroviral drugs. Viral sequencing studies show remarkable homogeneity in the infecting virus, with HLA class I genotype making a significant contribution to viral evolution (4).
Method: DNA samples from 261 HIV-1 infected and 252 uninfected individuals were typed for 15 *KIR* genes by PCR-SSP (sequence specific priming). HLA typing was performed using both PCR-SSP and sequencing techniques.
Results: Preliminary analysis shows that most inhibitory *KIR* genes are present in more than 90% of the study population while the frequencies of activating *KIR* genes ranged from 11% to 38%. Potentially new polymorphisms were detected in certain KIR loci including 3DL1/S1 and 2DL2 that warrant further investigation.
Conclusion: The frequencies of most activating *KIR* genes were significantly lower compared to those of their corresponding inhibitory counterparts. Analyses of KIR-HLA compound genotypes will be presented.

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Susceptibility to secondary bacterial complications following influenza infection: the role of miRNA in TLR desensitisation

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Secondary bacterial infections are a dangerous consequence of an initial viral lung infection. Analysis of post-mortem specimens from the 1918 influenza pandemic and the recent swine-flu pandemic has shown that although many people were infected with these viruses, of those that died the vast majority had a secondary bacterial complication.

Using mice to model the dynamics of secondary bacterial susceptibility following influenza infection, we have found that susceptibility extends far beyond the period of acute viral disease. We believe that in part this is due to innate imprinting and a lowering of the threshold of activation of the innate immune rheostat.

The innate immune system of the airway is held in a state of heightened regulation compared to more sterile tissues so as to avoid unnecessary inflammation to innocuous antigen. Once a viral infection is cleared the airway attempts to return to a regulated, homeostatic state. As a result, there is a period of excessive regulation characterised by TLR desensitisation, and a high expression of negative regulators like CD200R on alveolar macrophage. We compared the transcriptome of alveolar macrophage from naïve mice with those from mice following a viral infection. We found highly significant differences between the miRNA profile of naïve and resolved macrophage. By manipulating these miRNA we were able to alter their TLR sensitisation thereby converting naïve macrophage into 'resolved' macrophage.

It is hoped that by determining the pathways involved in over-regulation of alveolar macrophage, therapeutic targets will be suggested, which may protect against secondary bacterial infections.

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Immunoglobulin light chain allelic exclusion in Systemic Lupus Erythematosus (SLE)

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Immunoglobulin gene rearrangement by B cells inevitably generates some rearrangements that are not productive; either they are in the incorrect genetic reading frame, or the resultant amino acid sequence may hold unfavourable properties. In healthy B cells, unwanted rearrangements of Ig kappa light chains are genetically inactivated by rearrangement of the kappa deleting element (KDE) to prevent the expression of two light chains by the same B cell (allelic exclusion).

RT-PCR assays reveal that KDE rearrangements are less frequent in SLE B cells compared to healthy controls. Sequence analysis demonstrates a significantly higher load of somatic hypermutations within non-productive rearranged kappa light chain alleles, which typically should have been silenced by rearrangement of the KDE, supporting the idea that KDE rearrangements are inefficient in SLE.

Failure to obey the rules of light chain allelic exclusion leaves the potential for the expression of two Ig light chains by some B cells. Distortions observed in the FACS profiles of SLE PBMCs stained for kappa and lambda light chains reveal that in SLE, B cells have both kappa and lambda light chains on their surface.

These observations are highly relevant in the context of SLE, since dual light chain expression could contribute to the polyreactive and frequently self-specific nature of SLE B cells.

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High-mobility group box 1 contributes to the initiation of autoimmune diabetes

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Type 1 diabetes is a result of the autoimmune destruction of theβ-cells within the islets of Langerhans, while the implication of the innate immunity has been proposed in recent years. High-mobility group box 1 (HMGB1), an inflammatory trigger in a number of autoimmune diseases, has been shown to activate pro-inflammatory responses when released by necrotic cells into the extra cellular milieu. Our aim was to investigate the possible significance of HMGB1 in the natural history of diabetes in non-obese diabetic (NOD) mice. The distributions of HMGB1 in the pancreas were examined using immunohistochemical staining. Here, we observed that the rate of HMGB1 translocation from islet nuclei to cytoplasm was rapidly increased in the diabetic mice. HMGB1 receptors on different pancreatic islets were further studied using confocal immunofluorescence microscopy. The cells positively stained for Toll-like receptor (TLR) 4 wereβ-cells, however, only a few α-cells were positively stained for TLR4. We further examined the effects of anti-TLR2, anti-TLR4 and anti-TLR9 antibodies on HMGB1 cell surface binding in islets, which indicated HMGB1 can interact with TLR4 in islets. The changes of HMGB1 and TLR4 were also detected by qRT-PCR and Western blotting during the whole course of diabetes. Both targets significantly upregulated in early-developed diabetic mice and slowly downregulated in the following weeks. Our study demonstrates that HMGB1 acts through TLR4 signaling to selectively damage the β-cells during the development of type 1 diabetes.

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Cross-talk between IKK2 and Notch promotes pancreatic cancer progression in mice through Hes1 mediated PPARγ inhibitio

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Activating mutations of Kras are found in more than 90% of pancreatic ductal adenocarcinomas (PDAC) and can result in increased activity of the NF-κB pathway, leading to a constitutive production of pro-inflammatory cytokines, such as TNF-α. Well-described mouse models with pancreas-specific activation of oncogenic Kras display the full spectrum of pancreatic intraepithelial neoplasias (PanINs) and recapitulate the major features of human PDAC. We used the *kras* $^{+/\text{LSL-G12D}}$; pdx1-cre model to determine the role of IKK2/NF-κB signalling in formation and progression of PanINs. We showed that genetic deletion of *ikk2* in *kras*$^{+/\text{LSL-G12D}}$; ikk2$^{f/f}$; pdx1-cre mice blocked the progression of PanIN lesions. We further demonstrated that TNF-α stimulation of initiated epithelial cells via IKK2 engaged with canonical Notch signalling to upregulate the expression of primary Notch target genes. The cross-talk between NF-κB and Notch downregulated *pparg*, a repressor of inflammatory gene expression and retained a constitutive production of pro-inflammatory mediators and cytokines by the transformed cells. Our findings reveal a malignant cell-autonomous, low-grade inflammatory process operating from the very early stages of Kras-driven pancreatic carcinogenesis which acts as a tumour promoter for PanIN lesions through Hes1 mediated inhibition of PPARγ.

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T cell anergy and immune tolerance patterns in formalin-fixed paraffin-embedded oral squamous cell carcinoma and metastatic lymph nodes

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Background: Oral squamous cell carcinoma (OSCC) develops in an immune cell-rich environment, where inflammatory cells in the tumour microenvironment establish an anti-tumour response by secreting pro-inflammatory cytokines. The cancer cells also can induce various mechanisms suppressing the anti-tumour response such as regulating network of suppressive cytokines and recruitment of suppressive regulatory T cells (Tregs). These escape mechanisms are seen at the local tumour site and similar mechanisms may also occur in regional lymph nodes (LN).
Objectives: To investigate T cell anergy and immune tolerance patterns using immunofluorescence and gene expression analysis in formalin fixed paraffin embedded (FFPE) tissue from primary OSCC tissues and metastatic LN.
Methods: Archival cases of OSCC were stained via single and/or double immunofluorescence with T cell anergy receptor markers including Foxp3, Foxp3/CD4 and foxp3/CD8 antibodies. For investigation of gene expression a further 38 FFPE archival cases of OSCC were divided into three groups: (i) primary OSCC without metastasis (ii) OSCC with associated metastatic LN and (iii) control tissues. The expression of human T cell anergy and immune tolerance genes was determined using focused array technology via RT-PCR.
Results: A higher frequency of Tregs and T cell anergy markers including Fas were observed on the lymphocytes of OSCC compared to controls. Preliminary data on genes expression showed that increased expression of FoxP3 genes can be observed in primary OSCC and LN.

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Hsp-27 prolongs cardiac allograft survival by protecting from apoptosis and by delaying infiltration

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Previous studies have suggested that over-expression of hsp-27 protects against atherosclerosis and cardiac allograft vasculopathy in humans and *ex-vivo* induced ischemic damage in murine. The purpose of this study was to determine whether over-expression of hsp-27 protects the heart from acute rejection. B10.A mice, over-expressing Ha-tagged human hsp-27 were used as donors (Tg). Tg hearts and wild-type hearts were exposed to ischemia *ex-vivo* and the extent of apoptosis was determined using TUNEL assay and estimation of Caspase-3, Caspase-9 and Caspase-1 activities. The increase in apoptotic cells as well as Caspase-3 and Caspase-9 activity in response to ischemia was significantly reduced in transgenic hearts (1.53; 1.84 and no fold increase respectively) compared to wild-type (2.56; 2.60 and 2.0 fold increase respectively). In contrast, Caspase-1 activity following ischemia was similar in both Tg and Wt hearts (130% increase). Interestingly, Caspase-1 activity was significantly reduced in Tg normoxic heart compared its littermate control ($P < 0.01$). B10.A hearts from Tg or wild-type controls were transplanted into C57BL/6 wild-type recipients, representing a complete mismatch. Daily palpation of the transplanted hearts revealed significantly prolonged cardiac allograft survival of Tg hearts (35 ± 10.37 days, $n = 10$) compared to wild-type controls (13.6 ± 3.06 days, $n = 10$, $P = 0.0004$). Prolonged allograft survival was accompanied by significant changes in infiltrating cells (CD4+ T-cells and monocytes) as well as cytokine production (IFN-gamma and IL-4). The data so far suggest that hsp-27 may delay acute allograft rejection by limiting ischemia-induced apoptosis and delaying infiltration of inflammatory cells.

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Does macrophage activation influence wound healing?

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Chronic non-healing wounds in the elderly population are associated with substantial morbidity and mortality and impose a significant financial burden upon the world's health services. Chronic wounds are characterised by a prolonged and excessive inflammatory response which is widely hypothesised to impede wound healing. Conversely, recent murine studies have revealed that macrophages are required at the early stage of healing, with targeted macrophage ablation delaying wound repair. Macrophages can be polarised to classical or alternative activation phenotypes, primarily in response to specific wound cytokines. Alternatively activated macrophages are 'pro-healing' and act to counter-balance 'pro-inflammatory' classically activated macrophages. Here we report the temporospatial profile of classical versus alternative macrophage activation during acute wound healing and contrast this with an age-associated delayed healing model. Intriguingly, macrophage polarisation predicts healing outcome. To functionally explore the role of alternatively activated macrophages we have modulated the function of arginase, a validated AA marker and an enzyme with an important role during healing. Pharmacological inhibition of arginase activity directly perturbed healing, but interestingly did not influence the absolute numbers of alternatively activated macrophages. We propose that arginase is critical for healing and may represent an important therapeutic target.

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Clinicopathological expression of CD133 in colorectal cancer: Malaysian experience

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The incidence rates of colorectal cancer (CRC) among economically transitioning countries are continuing to increase. Along with the worldwide variation in the incidence patterns, newer biological markers were identified and used as diagnostic and prognostic tools in particular the cancer stem cells (CSC) glycoprotein. This fast moving trend of defining the importance of CSC markers is, however yet to be explored in Malaysian CRC patients. Hence, a retrospective study was undertaken to investigate the expression of CD133, a CSC cell-surface marker in CRC specimens of patients admitted to UKM Medical Center. An immunohistochemical examination of CD133 expression and a clinicopathological analysis were conducted in the 120 CRC patients. The pattern of CD133-positive cells was evident at the glandular-luminal surface of the epithelial tumor cells in all of the cases examined. While there was no significant association between genders, a statistical significant difference was obvious among the ethnic groups in which the Chinese exhibited a prominent expression of CD133 ($P = 0.002$). Higher intensity, an indicative of strong expression of CD133 was detected in cases diagnosed as poorly-differentiated colorectal carcinoma ($P = 0.007$). Similar frequency of CD133 expression was noted in Duke's B patients which was statistically significant ($P = 0.046$). The results indicate that the detection of CD133 by immunohistochemistry may facilitate *in situ* characterization of CRC in this region. These data emphasize the importance of CSC markers to be considered as an effective prognostic factor.

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The autoimmune-protective *IL23R* A/Glu^{381} allele promotes IL-23 unresponsiveness in human memory T helper 17 cells

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The minor, non conserved allele, Glu^{381}, of the R381Q single nucleotide polymorphism (rs11209026G>A; population frequency = 0.056) of the IL-23 receptor gene (*IL23R*) has been consistently reported to protect against immune-mediated common complex diseases. Nevertheless, the biological effect of carrying this variant has not been determined. We have recently shown impaired IL-23-induced IL-17A production and STAT3 phosphorylation in Th17 cells generated *in vitro* from healthy individuals heterozygous for the protective A allele (GA). Here, we took advantage of the large Cambridge BioResource of volunteers to expand our functional investigation of the *IL23R* R381Q gene variant in a cohort of healthy individuals, which included ten individuals homozygous for the protective A allele (AA) even though they comprise only 0.36% of the Cambridge population. By using isolated memory CD4+ T cells, and in keeping with our previous study, we found attenuated IL-23-induced Th17 response in heterozygous individuals. Moreover, we found that AA homozygous individuals were strikingly unresponsive to IL-23, with minimal or no IL-17A and IL-17F production and failure of memory Th17 cell expansion. Taken together, our data provide evidence for an allele dosage effect for IL-23R Glu^{381} and indicate that common gene alleles associated with complex diseases might have biological effects of considerable magnitude in homozygous carriers. Moreover, our study paves the way for larger scale studies in individuals with disease and at high risk of developing the disorder, aimed to translate insights gleaned from 'gene-to-function' studies in the healthy population to better understanding of disease pathogenesis and stratified medicine approaches.

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HIV infection alters pneumococcal-specific Th1 and Th17 responses

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Background and aims: In HIV infection, *Streptococcus pneumoniae* is an important upper respiratory tract pathogen with higher rates of nasopharyngeal colonization and invasive disease. Data suggests that two lineages of CD4 T cells facilitate the clearance of pneumococcal colonisation (Th1 and Th17) and control bacteria following dissemination (principally Th1). We aimed to investigate whether pneumococcal-specific Th1 and Th17 responses differentially wane as HIV progresses and contributed to increased colonization.
Methods: Nasopharyngeal swabs and peripheral blood was obtained from 100 HIV-infected Malawian adults during different stages of HIV infection. DNA was isolated from swabs and microarray used to identify the carriage of invasive and non-invasive serotypes during HIV infection. Peripheral blood mononuclear cells were stimulated with pneumococcal-antigens and Th1 IFN-γ and Th17 IL-17 responses were evaluated using a combined proliferation and intracellular cytokine assay by flowcytometry.
Results: Pneumococcal carriage increases as CD4 counts decline and individuals progress to WHO stage IV symptomatic disease (HIV$^-$13% versus HIV$^+$35%), colonisation remains high in those virally suppressed by anti-retroviral therapy (ART) (33%). HIV-infected individuals carry a broad range of invasive (HIV$^-$5 versus HIV$^+$15 serotypes) and non-invasive (HIV$^-$6 versus HIV$^+$12 serotypes) as compared to HIV-negative controls. Changes in nasopharyngeal pneumococci was associated with selective decrease in pneumococcal-specific IFN-γ responses but preserved IL-17 production, however overall, there were less responders due to CD4 T cell depletion and impaired proliferation.
Conclusions: The startling rise in carriage rates even in those established on ART helps maintain a reservoir for pneumococcal transmission and increases the chance of bacterial invasion.

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The role of the pattern recognition receptor Nod2 in cutaneous wound healing

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Chronic wounds lead to substantial morbidity and mortality and are a financial burden to the global healthcare economy. While poor progression of a chronic wound is often associated with the presence of a biofilm infection, our understanding of wound biofilm formation and diversity is very limited. Biofilms are diverse poly-microbial communities, consisting of both gram-positive and negative bacteria, that are found throughout nature. Innate host response mechanisms have evolved whereby potentially harmful pathogens are recognised by multiple host pattern recognition receptors (PRRs), found either on cellular membranes or in the cytosol. Their co-ordinated activation via known signalling pathway results in the induction of pro-inflammatory cytokines. NOD2, a cytoplasmic PRR has been strongly implicated in chronic inflammation of the gut, where loss-of-function mutations have been linked to Crohn's disease. In the small intestine, Nod2 has been suggested to control the host's response to commensal bacteria, however the function of Nod2 in the skin remains poorly understood. Here we demonstrate an important role for Nod2 in skin wound healing. In the absence of Nod2 healing is substantially impaired with perturbed re-epithelialisation and increased inflammation. These findings begin to the address the mechanisms by which local micro-flora may influence skin wound healing.

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KIR expression as a biomarker of multiple sclerosis disease type

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Multiple Sclerosis (MS) is a neuroinflammatory disease of the central nervous system, characterised by a heterogeneous patterns of disease activity ranging from a relapsing-remitting (RR-MS) to progressive (P-MS) disease course. Both adaptive and innate immune cells are thought to play a role in disease. There is evidence for involvement of both NK and iNKT cells, including immunogenetic data indicating that killer immunoglobulin-like receptor (KIR) genotype influences susceptibility. We assessed at a cellular level the frequency of different classes of KIRs by means of multiparameter flow cytometry. We established a nine-colour FACS panel to investigate KIR expression on both NK cells, CD56negative NK cells and iNKT cells. Cohorts of healthy donors, P-MS, untreated RR-MS, patients treated with beta-interferon or Tysabri, as well as patients with clinical isolate syndrome (CIS) were recruited. While there was no difference in the absolute frequency of NK cells between the different groups, NK cells expressing KIR2DL2/L3 and KIR3DL1 were significantly reduced in untreated RR-MS patients compared to controls. Treatment with either beta-interferon or Tysabri was associated with increased expression of both KIRs. P-MS and CIS patients showed higher levels of KIR2DL1/S1 than healthy donors and RR-MS patients, suggesting a different stage in the immunological process. Interestingly, expression of KIR3DL1 is virtually absent on iNKT cells in untreated RR-MS patients. Studies are underway to correlate these findings with HLA genotype and KIR allelic variant and copy number genotype.

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Leukocyte expression of inflammatory cytokines, heat shock proteins and adiponectin in osteoarthritis-afflicted type 2 diabetics

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Aims: The present study profiles peripheral blood CD3+ T lymphocyte subpopulations contributing to T2DM-associated osteoarthritis (OA), hemeoxygenase-1 (HO-1) a major cytoprotective enzyme; heat shock proteins (HSP) 70 and 90 which maintain cell viability; and adiponectin, a glucose- and fatty acid-regulatory hormone.
Methods: Peripheral blood mononuclear cells from 15 T2DM-OA patients; 15 non-OA T2DM patients; and 10 healthy control subjects were evaluated by two-color flow cytometry for representation of CD3+ T cells producing interferon-gamma (IFN-Y, tumor necrosis factor alpha (TNF-α); ineterleukin-6 (IL-6) and ineterleukin-1beta (IL-1β). HO-1, HSP70, HSP90 and adiponectin were assayed using Enzyme-Linked Immunoassay.
Results: Non-OA T2DM blood did not contain significantly increased inflammatory T cell phenotypes versus control blood. However, CD3+IFN-Y and CD3+TNF-α cells were elevated in T2DM-OA blood relative to controls ($P < 0.05$); and to non-OA afflicted diabetics ($P < 0.05$). HO-1 and adiponectin expression in blood from both OA and non-OA T2DM patients was significantly lower than in healthy subjects ($P < 0.05$); and HSP 70 was elevated in OA-afflicted diabetics relative to controls ($P < 0.05$), however non-significant differences were noted in these metabolites in OA versus non-OA diabetic blood. Finally, Pearson product-moment analysis revealed significantly inverse correlation between HO-1 and TNF-α ($r = -0.886$, $P = 0.008$); HO-1 and IL-1β ($r = -0.923$, $P < 0.009$); and adiponectin and TNF-α ($r = -0.748$, $P = 0.033$) in OA-T2DM subjects.
Conclusions: The present study demonstrates correlation between potentially pathogenic T cell phenotypes and regulators of diabetes-associated response to oxidative stress: HO-1 and adiponectin. These outcomes contribute to improved pharmacological strategies for modulating these mediators.
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CD11c positive cells are critical for the maintenance of Th2 responses and survival during chronic helminth infection

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Dendritic cells (DCs) are key players in induction of immune responses through their ability to activate naive T cells, but are thought to be less necessary for maintenance of T cell effector function. We have previously demonstrated that CD11c positive cells are necessary for priming of the early Th2 response to the medically important parasitic helminth *Schistosoma mansoni*, using CD11c.DTR mice which allow depletion of CD11c positive cells including conventional (70–80% depletion) and plasmacytoid (80–90%) DCs. In the current study we have gone on to deplete CD11c positive cells at later stages of *S. mansoni* infection, from a time point where the immune response has been on-going for 3–4 weeks, and immunopathology is evident. At this stage of infection, cross-talk between the immune response mounted against both the helminth pathogen and commensal bacteria is thought to be significant, and important in determining the severity of inflammation and pathology that develops. Surprisingly, depletion of CD11c positive cells at this chronic stage of infection resulted in dramatically impaired Th2 cytokine production, coincident with severe weight loss. Our data point to an unexpectedly important role for CD11c positive mononuclear phagocytes in the maintenance of CD4 T cell responses and regulation of pathology during chronic helminth infection, and ongoing work is aimed at identifying the mechanism(s) underlaying the defective Th2 response and exacerbated morbidity in CD11c depleted animals and the possible involvement of commensal bacteria in this process.

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The immunopathogenesis of ankylosing spondylitis: a dendritic cell perspective

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Ankylosing Spondylitis (AS) is a multi-organ, chronic inflammatory disease. The axial skeleton, peripheral joints and intestine are prominent sites of inflammation in AS. These symptoms cause loss of joint function and severe disability. Another hallmark of AS is the strong genetic association of the MHC class I molecule, HLA-B27. However, mechanisms underlying HLA-B27 involvement with disease progression remain elusive.

Dendritic cells (DCs) are essential for maintaining homeostasis between protective immunity and tolerance. Consequently, DCs are implicated in AS pathogenesis. Rats expressing the human HLA-B27 and $\beta 2$ microglobulin transgenes develop systemic intestinal and peripheral disease symptoms, similar to those observed in AS patients. Use of this animal model has enabled investigation into the role of DCs in disease development. Our results show that B27-TG rats lack the tolerogenic $CD103^+$ MHC II^+ $CD172\alpha^{lo}$ intestinal DC subset. This DC defect is accompanied with a reduction in plasmacytoid DCs (pDCs) and a deficiency in the generation of bone-marrow derived DCs. Overall, these results indicate that deficiencies in DCs may promote disease development.

We have now investigated DCs in the blood from AS patients, to understand whether they display similar defects, and have identified several differences in the proportions of human DC subsets between AS patients and healthy controls. We observe a trend towards reduced frequency of $CD141^+$ $CD11c^+$ DCs in AS patients, and a significant loss of pDCs ($CD123^+$ $CD304^+$). Future investigations will focus on understanding the impact of these DC deficiencies in AS pathogenesis.

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Synergistic influence of tapasin and HLA class I protection against chronic hepatitis C virus infection

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Specific HLA class I alleles are associated with the outcome of hepatitis C virus (HCV) infection. Tapasin is part of the peptide loading complex and is thought to optimise the peptide repertoire of specific tapasin-dependent HLA-class I alleles. The SNP rs2071888 is a non-synonymous G/C polymorphism in exon 4 of the *Tapasin* gene causing an arginine to threonine substitution. We hypothesized that polymorphisms in the tapasin gene could affect clearance of HCV in combination with specific tapasin-dependent HLA alleles.

Two hundred and sixteen chronically infected individuals and 120 spontaneous resolvers of HCV were genotyped for rs2071888 and for HLA class I. The association of these results with the outcome of HCV infection was determined.

The tapasin-G allele was associated with resolution of HCV infection ($P = 0.018$, OR = 1.99, 95% CI = 1.14−3.46). Interestingly, tapasin heterozygosity in combination with heterozygosity at HLA-B was also protective (P-trend = 0.005). Furthermore, we identified specific HLA-B alleles associated with protection in the context of Tapasin G. The G allele was most protective in combination with HLA-B*0702 ($P = 0.029$, OR = 4.56, 95% CI = 1.2−17.27), and HLA-B*5701 ($P = 0.029$, OR = 12, 95% CI = 1.2−120). Tapasin dependence is determined by amino acids 114 and 116 of HLA class I. Consistent with this we found that aspartate at position 114 and serine at 116 were protective against chronic HCV infection in the context of the G allele (D114/TapG, $P < 0.0001$, OR = 3.3, 95% CI = 1.83−5.98; S116/TapG, $P < 0.0001$, OR = 2.73, 95% CI = 1.57−4.76).

Our data demonstrate that tapasin polymorphism can be an important factor in the successful resolution of HCV infection.

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NKT cells aggravate the development of abdominal aortic aneurysms

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Abdominal aortic aneurysm (AAA) is a dilatation of the abdominal aorta and is mostly undiscovered until it ruptures leading to serious complications and mostly to death. Development of AAA is associated with an accumulation of inflammatory cells in the lesions such as NKT cells. The exact contribution of these cells in AAA formation remains unclear. The goal of this study was to investigate the role of NKT cells in angiotensin II (AngII) induced AAA formation.

To investigate the influence of AngII on NKT cell activation, NKT hybridoma cells were cocultured with DCs which were incubated with AngII in combination with α-GalCer. AngII amplified the α-GalCer induced activation of NKT cells, observed by increased production of IL-2 by the NKT cell hybridoma. In addition, AngII increased IFN-γ production by α-GalCer activated splenocytes. To investigate the role of NKT cells in AAA formation, $LDLr^{-/-}$ and $LDLr^{-/-}CD1d^{-/-}$ mice were fed a Western-type diet prior to infusion with AngII. Five out of 12 $LDLr^{-/-}$ mice died due to rupture of the aorta while in the $LDLr^{-/-}CD1d^{-/-}$ group no mice died. A clear significant difference in severity of AAA was observed in the surviving mice. In seven out of 11 $LDLr^{-/-}CD1d^{-/-}$ mice no lesions were found compared with only one out of 12 $LDLr^{-/-}$ mice.

These data show that NKT cells aggravate the development of AngII-based AAA and aortic ruptures possibly due to an indirect effect of AngII on NKT cells. These results provide new opportunities to intervene in the development of aneurysms.

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Killer cells in asthma with fixed airflow obstruction

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The majority of asthma cases display complete airflow reversibility. However, recent attention has increasingly focussed on a small subset of asthma patients that display fixed airflow obstruction (FAO) despite optimal treatment, characteristic of COPD. A growing amount of literature has linked the three main types of killer cells, namely CD8 T cells, Natural Killer (NK) and NKT cells, to both traditional asthma and COPD due to their cytotoxic and immunoregulatory functions. We aimed to study the role of these cells in asthma with FAO. Absolute cell counting, cytotoxic mediator and receptor profiling, activation time course assays as well as functional cytotoxic experiments were performed to compare the number and function of killer cell subsets in the peripheral blood of asthma patients with and without FAO and healthy controls. Our data suggest both the number and cytotoxic function of these killer cells are reduced in the peripheral blood of asthma patients with FAO, as quantified by functional cytotoxic assays. This indicates killer cells may be recruited to the lung in patients with FAO and have a subsequent role in the disease pathogenesis, as has been shown in COPD.

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Lower expression of miR-155 in asthmatic compared to healthy airway smooth muscle

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The phenotype of airway smooth muscle (ASM) is altered in asthmatic patients compared to healthy subjects. Hyperplasia, hypertrophy, increased contraction and altered synthetic capabilities of ASM cells have all been reported. Despite the evidence demonstrating an altered ASM phenotype in asthma the molecular mechanisms underlying these changes remain unclear. This project investigates the potential role of microRNAs (miRNAs) in contributing to the asthmatic phenotype by comparing their expression levels in healthy and asthmatic ASM.

miRNA expression profiling was performed on cultured ASM cells isolated by explant culture from endobronchial biopsies of three healthy and three moderate asthmatic volunteers. This analysis identified 40 miRNAs with a >2-fold difference in expression between healthy individuals and moderate asthmatics. In particular we have determined, by both microarray and qPCR validation studies, that miR-155 expression in asthmatic ASM is approximately half that of healthy ASM. We have also shown that the miR-155 housing gene, *BIC*, is expressed at a lower level in asthmatic ASM. This data is consistent with a miR-155 knock-out mouse model, which spontaneously develops lung remodelling similar to that observed in asthma, including an increase in ASM mass. These data therefore suggest that a decrease in miR-155 expression may have potentially pro-asthmatic functions in ASM.

Transcriptome profiling of cultured ASM cells following both overexpression and antagonism of miR-155 has identified a number of potential targets for miR-155. These include a variety of transcription factors and immunomodulatory molecules that may have roles in promoting a pro-asthmatic phenotype.

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A hyper-IgE syndrome mouse model

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Dominant-negative mutations in the transcription factor signal transducer and activator of transcription 3 (*STAT3*) have been shown to be causative for the multi-system disorder hyper-IgE syndrome (HIES). However, deciphering STAT3's precise role in disease pathogenesis is hampered by the lethality associated with germline deletion of Stat3 and the severe abnormalities associated with tissue-specific deletion. To clarify potential mechanisms, we generated bacterial artificial chromosome (BAC)-transgenic mice that expressed a HIES-associated *Stat3* allele. The mutant allele was a deletion of valine 463 in the DNA-binding domain and was expressed at equivalent levels to that of wild-type Stat3 in our model. Transgenic cells exhibited normal tyrosine phosphorylation of Stat3 following acute cytokine stimuli but marked inhibition in DNA-binding activity. These mice also had elevated serum IgE levels and showed a partial deficiency in IL-17 production. Collectively, the dominant-negative action of the mutant transgene, the elevated IgE levels and the IL-17 defect mirrored the HIES clinical phenotype, strongly supporting our system as a useful mouse model of this disease.

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Effect of the immune system in the pathogenesis of staphylococcal mastitis in rabbits. Preliminary results

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Staphylococcal mastitis is the main cause of culling of adult does from commercial rabbitries. However, scarce information is available on the pathology and pathogenesis of mastitis in rabbits. The aims of this work were

1 To provide a detailed description of the spectrum of microscopic pathology in cases of chronic staphylococcal mastitis in adult does;

2 To determine the possible correlation between *Staphylococcus aureus* genotypes and pathology and

3 To characterize local and peripheral immunity in order to deep into the pathogenesis of this important mammary infection.

Ninety adult rabbits (*Oryctolagus cuniculus*) from rabbitries with previous diagnosis of chronic mastitis were studied. Next analyses were carried out on each animal:

1 Genotyping of *S. aureus* strains isolated from lesions,

2 Study of lymphocyte populations in peripheral blood by flow cytometry,

3 Histopathologic classification of mammary lesions, and

4 Analysis of local immune response by immunohistochemical studies in mammary glands and periglandular tissue.

On the basis of histopathology, pathological changes were differentiated into abscesses, suppurative mastitis with lobular pattern, cellulitis and mixed lesions. These different pathological presentations were independent of *S. aureus* genotype. There were differences among lesions regarding cells populations. The number of T and B lymphocytes decreased with the maturation of the abscesses, while the number of plasmatic cells, macrophages and B lymphocytes rose as the lesion spread. A broad spectrum of pathological states could be established based on the histomorphological characteristics and the cellular composition of the lesions, which may reflect different via of infection and host-pathogen interactions.

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Exacerbated hematopoietic stem and progenitor cell activity during intestinal inflammation

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Pathogenic Th1/Th17 responses that occur during colitis correlate with a sustained accumulation of short-lived neutrophils and inflammatory monocytes in peripheral lymphoid organs and the colon, putting significant pressure on the supply of innate immune cells from the bone marrow (BM). However, the upstream regulation of the rare hematopoietic stem cells and progenitor cells is unknown during colitis and most chronic inflammatory diseases. In this study we showed that hematopoiesis was skewed toward granulocyte-monocyte progenitor (GMP) production during colitis at the expense of erythroid and lymphoid progenitors, along with a striking accumulation of haematopoietic stem cells (HSC) in the BM. These profound changes in the BM were accompanied by extramedullary hematopoiesis as HSCs and GMPs accumulated in the spleen of colitic mice. Surprisingly, clonogenic GMPs also accumulated in the intestinal mucosa and this correlated with the emergence of a Colony Forming Unit activity (CFU) in the colon.

Dampening the excessive HSC proliferation and tipping the progenitor cell production toward a normal leukocytic balance could constitute a new therapeutic strategy for the treatment of inflammatory bowel disease.

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Evaluation of LIVIN protein expression, as promising marker in infiltrating background and malignant cells of Hodgkin Lymphoma, compared to non-neoplastic lymph node

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Background: A novel human inhibitor of apoptosis protein (IAP) family member termed Livin, was demonstrated in pathogenesis of different human malignancies, and also is being investigated as potential treatment targets in cancer patients. However there is no report on Livin expression in Hodgkin Lymphoma.
Method: In this study, we evaluated Livin expression in 78 paraffin embed block including evaluated Livin expression in 39 staged cases of HL in comparison with 39 control subjects (normal and reactive hyperplasia lymph nodes) which are randomly selected. Tissue Microarray-based Semi-quantitative Immuno-flourecent Staining was applied for protein expression profiling in control subjects and also both infiltrating non-neoplastic cells (preferentially Lymphocytes) and neoplastic cells (Hodgkin and Reed-Sternberg) of cases.
Result: At this study the mean ratio of Livin/GAPDH expression was significantly increased between infiltrating background cells in hodgkin Lymphomas and control cases (0.54596 versus 0.50827, $P < 0.001$). Also a significant difference was found in mean ratio of Livin/GAPDH expression between neoplastic cells (HRS) and major background cells in tumor microenvironment (0.59024 versus 0.54596, $P < 0.001$), Furthermore, this study confirmed significant increase of livin expression in Early-stage toward Advanced-stage in HL (0.52888 versus 0.580146, $P < 0.01$).
Conclusions: These findings suggest that the Livin may have critical role in the pathogenesis of Hodgkin lymphoma and also could be a novel prognostic marker in this kind of lymphoma. In summary, Livin can be regarded as a promising target for experimental anticancer therapy in patient with HL.

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Mannan-binding lectin concentration in children with glomerulonephritis

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Background: Mannan-binding lectin (MBL) is considered an important component of innate immunity and its insufficiency appears to be a significant risk factor for infections in infants, for individuals of any age undergoing chemotherapy or post-transplantant immunosuppression. Low MBL level suppose to associate with immune-mediated diseases like systemic lupus erythematosus (SLE), rheumatoid arthritis etc.

The aim of this study was to investigate the MBL concentration in children with SLE and Acute Post streptococcal Glomerulonephritis (APSGN).
Methods: Fifty children with APSGN and 15 children with SLE were prospectively investigated for the MBL level by ELISA and compared to 40 healthy controls.
Results: All healthy controls had MBL concentration above 1000 ng/ml whereas 17 of 50 children with APSGN (34%) were found to have MBL ≤ 1000 ng/ml ($P < 0.05$) and 8 (16%) had MBL ≤ 500 ng/ml ($P < 0.05$). Fifty percent patients with SLE had low MBL level ($P < 0.05$). Low serum MBL concentrations were not correlated with more severe manifestations of APSGN whereas MBL-hypocomplementemia in patients with SLE was found to be associated with disease activity (66.7%) and with diffuse-proliferative forms of lupus nephritis (class IV) (41.6%).
Conclusions: There was not found the correlations between low MBL level and severity of APSGN. MBL-hypocomplementemia in patients with SLE was associated with more active and severe morphological diseases.

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Host defense peptide LL-37 induces release of eicosanoids and eosinophil cationic protein from human eosinophils – implications for asthma

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Background: The host defense peptide L-37 exhibits various immunomodulatory activities. However, its role in eosinophil-associated asthma is unclear. Eosinophils and their products including eicosanoids and eosinophil cationic protein (ECP) are key mediators of inflammation and tissue damages in asthma.
Objective: We sought to investigate the clinical implications of LL-37 in eosinophilic asthma.
Methods: Primary eosinophils were isolated from peripheral blood of healthy volunteers and asthma patients. Eicosanoid and ECP levels were measured using specific EIAs or ELISAs. Expression and activation of eicosanoid-synthesizing enzymes and signaling kinases were analyzed by Western blot or Immunofluorescent staining. LL-37/hCAP18 expression was analyzed by Western blot.
Results: LL-37, via formyl peptide receptor-2 (FPR-2), triggered the release of eicosanoids mainly cysteinyl leukotrienes (cys-LTs) and thromboxane $(TX)A_2$ from eosinophils. The response was more prominent when the cells were primed under conditions mimicking asthma. Notably, LL-37 induced formation of the functionally active lipid bodies in eosinophils. For efficient eicosanoid synthesis, LL-37 induced intracellular mobilization and assembly of eicosanoid-synthesizing enzymes $cPLA_2$, 5-LO and LTC_4S, at both lipid bodies and perinuclear locations. Additionally to eicosanoids, LL-37 also induced the release of cytotoxic granular protein ECP, from eosinophils. Furthermore, leukotrienes could trigger rapid release of hCAP18, the proform of LL-37, forming a positive feedback regulation. Comparing healthy and asthmatic subjects, hCAP18 expression in eosinophils was enhanced in asthmatics, suggesting its positive correlation with the condition.
Conclusion: This study indicates the clinical relevance of LL-37 in eosinophilic asthma and suggests LL-37/hCAP18 as a correlative marker and potential therapeutic target for the condition.

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The analysis of anti- CCP antibodies and its activation in patients with arthritis rheumatoid

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Introduction: Rheumatoid Arthritis (RA) is a chronic systemic autoimmune disease that causes inflammation, pain, stiffness and destructive changes in the joints. Rheumatoid Factor (RF) has been the primary blood test used to detect RA.

To facilitate diagnosis during the early stages of the disease, when often not all clinical symptoms are manifest, a good serological marker is needed. Antibodies directed to citrullinated proteins provide this ability. The most sensitive assay to detect these antibodies is the so-called anti- cyclic citrullinated peptide (CCP) enzyme – Linked immunosorbent assay (ELISA) assay. In this search, the diagnostic and prognostic potential and the general utility in clinical practice of anti-CCP Antibodies are discussed. The Anti- CCP antibodies detection test is a relatively new assay to detect the citrulline antibodies in blood. These auto antibodies are produce by immune system in response to a perceived threat of citrulline, an α-amino acid produced from arginine in the citrullination process. The objective of this syudy was to investigate the presence and prediction value of Anti- CCP in RA patients and evaluate its sensitivity and specificity comparing to that of classic laboratory tests, CRP and RF.

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Analysis of the role of galectin-3 in the immune response to *Helicobacter pylori*

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Helicobacter pylori colonises more than 50% of the world's population and is the most prevalent cause of peptic ulceration and gastric adenocarcinoma. *H. pylori* persists in its host by subversion of the immune response to its own benefit. Galectin-3, a protein with immunomodulatory roles, has previously been shown to be secreted by gastric cells in response to *H. pylori* adhesion and to bind *H. pylori* O-antigen. This study investigates the potential role of the secreted galectin-3 in the modulation of host response against *H. pylori*. Results from this study have shown that co-incubation of DC-SIGN-Fc with galectin-3 in a solid phase DC-SIGN-Fc adhesion assay inhibits DC-SIGN- *H. pylori* O-antigen interaction in a concentration dependent manner. The interaction between *H. pylori* O-antigen and DC-SIGN has previously been shown to modulate dendritic cell maturation, suggesting that blocking of this interaction by galectin-3 may play a critical role in modulating the adaptive immune response to *H. pylori*. Additionally, this study has shown that the presence of *H. pylori* O-antigen increases intracellular survival in THP-1 cells, further suggesting that O-antigen and its interaction with innate immune receptors such as DC-SIGN and galectin-3 play an important role in modulating the immune response to *H. pylori*. Currently, monocyte-derived DCs are being used to investigate the immunomodulatory effects of extracellular galectin-3 on the interaction between cell surface expressed DC-SIGN and *H. pylori* O-antigen.

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The adoptive transfer of myeloid-derived suppressor cells modulates tumor growth in Urethane-induced lung cancer

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Myeloid-derived suppressor cells (MDSC) have been associated with tumor growth, poor prognostic in cancer patients and impaired response to anti-cancer therapies. We evaluated whether the increase in MDSC causes enhancement in tumor growth and the associated mechanisms. BALB/c mice were injected with Urethane (UR) to induce lung nodules and after 1 week they were adoptively transferred with enriched MDSC: CD11b+Gr-1+ (1×10^6). Four months later we evaluated the presence of lung nodules and MDSC in spleen and lung. Groups: Control, MDSC, UR, and UR+MDSC. Control and MDSC groups presented no lung nodules development whereas UR and UR + MDSC showed respectively 2–4 and 3–8 nodules. Nodules area was increased in UR + MDSC (0.06–0.9 mm) when compared with UR (0.008–0.057 mm). In spleen the median of MDSC was 0.9% in Control, 4% in UR, 3.3% in MDSC, and 1.9% in MDSC + UR. The median of MDSC in lung was 0.7% in Control, 2.8% in UR, 9.8% in MDSC and 5.0% in MDSC + UR. We concluded that UR injection causes increase in the percentage of MDSC in lung whereas the adoptively transfer of enriched MDSC: CD11b+Gr-1+ in the presence or absence of UR injection causes even higher percentage of these cells in lung suggesting cell migration. MDSC only was not able to cause lung nodules whereas lung nodules number and area were enhanced in MDSC + UR indicating that these cells potentiate lung tumor development in the presence of a carcinogen. Lung nodules development in MDSC+UR was associated with decreased percentage of MDSC in spleen and lung.

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A Th17-related cytokine profile in patients with dengue correlates with the severity of infection

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Introduction: Th17 cells play a key role in the pathogenesis of autoimmune diseases and mediate both: detrimental or protective roles during the infection by some pathogens. The role of Th17 cells and the cytokines produced by this T cell population in dengue infection have not been demonstrated previously.
Materials and methods: To further investigate the role of Th17-related cytokines in the pathogenesis of dengue, we measured Th17-associated cytokines in serum specimens from Mexican patients with acute infection. A total of 117 serum samples from patients with Dengue fever (DF) or Dengue Hemorragic (DHF) and 78 serum samples from healthy donors were analyzed by ELISA method.
Results: Very low levels of some Th17-related cytokines were detected in the serum of healthy controls (IL-17A = 16.1 pg/ml, IL-22 = 10.4 pg/ml and IL-23 = 15.6 pg/ml) whereas IL-17F and IL-21 levels were undetected.In contrast, high levels of these cytokines were found in patients with dengue hemorrhagic fever (IL-21 = 546.8 pg/ml, IL-17A = 156.2 pg/ml IL-22 = 56.4 pg/ml and IL-23 = 52.6 pg/ml) more than patients with dengue fever (IL-21 = 248.3 pg/ml, IL-17A = 66.6 pg/ml, IL-22 = 38.8 pg/ml and IL-23 = 30.8 pg/ml). No difference was found between IL-17F serum levels in patients with DF (IL-17F = 30.6 pg/ml) and DHF (32.2 pg/ml).
Conclusion: Taken together, these results are the first evidence about a Th17-related cytokine profile in dengue infection. The presence of high levels of Th17-related cytokines during severe illness (DHF) more than mild disease (DF) suggests that these cytokines may contribute to the immunopathogenesis of the viral disease.

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Development of a novel multiplex ELISA for the detection of bovine brucellosis

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Brucella abortus is the causative agent of bovine brucellosis, a worldwide bacterial zoonosis of significant economic and social importance.

Currently serological tests are the mainstay of diagnosis. However, they produce a problem with false positive serological reactors (FPSR) in areas of low prevalence. FPSR are caused by presence of antibodies raised against infection with bacteria possessing similar OPS structure, such as *Yersinia enterocolitica* O:9.

A multiplex serodiagnostic ELISA offers potential to eliminate FPSR, by combining data from multiple antigens to support the current *Brucella*-specific smooth LPS antigen ELISA. A serum sample may be tested against multiple antigens in a single well.

The multiplex format enabled simultaneous detection of antibodies to *Brucella*-specific smooth LPS antigen from *B. abortus* S99, *B. melitensis* 16M, rough LPS from *B. abortus* RB51, *Yersinia enterocolitica* O:9, native protein extract Brucellergene$^{TM;}$recombinant proteins BP26 and Lumazine Synthase.

A BioDot Inc. fluid dispensing system printed duplicate 25 nl spots of each antigen into a 96-well ELISA plate; a 14 spot array. The antigens detected anti-*Brucella* antibodies in serum and plates were read using a Q-view imager^{TM}; then signal intensity was calculated. To demonstrate utility of the multiplex ELISA a small panel of bovine sera were tested; including culture positives, negatives and FPSR.

In conclusion, a novel multiplex ELISA has been developed for the detection of anti-*Brucella* antibodies and offers much potential for the exclusion of FPSR by confirmatory testing. This technique also has potential for the future development of a serological assay for detection of human brucellosis.

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Immunoglobulin E-producing B lymphocytes mediate colitis in BALB/c mice

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Background and aims: Oxazolone-induced colitis is a T helper type 2-mediated disease which is analogous to human ulcerative colitis. In this study the distinct role of interleukin-4 receptor-alpha (IL-4Rα)-responsive T and B lymphocytes and their effector functions in oxazolone-induced colitis was investigated.
Methods: Previously characterized $CD4^+$ T cell-specific IL-4Rα-deficient mice (Lck^{cre}IL-4R$\alpha^{-/lox}$) and newly generated B cell-specific IL-4Rα-deficient mice ($mb1^{cre}$IL-4R$\alpha^{-/lox}$) were treated with oxazolone and monitored for disease symptoms.
Results: Lck^{cre}IL-4R$\alpha^{-/lox}$ mice were protected from disease correlating with reduced IL-4, IL-13 and immunoglobulin (Ig)E responses. Adoptive transfer of naïve wild type $CD4^+$ T helper cells depleted of NK T cells restored a susceptible phenotype. In contrast Lck^{cre}IL-4R$\alpha^{-/lox}$ mice remained protected by transfer of IL-13-deficient $CD4^+$ T cells, suggesting that disease onset is not limited to natural killer T cell functions but critically depends on IL-13 production by $CD4^+$ T helper cells. Furthermore, $mb1^{cre}$IL-4R$\alpha^{-/lox}$ mice, unable to produce IgE, were also protected from colitis. *In vivo* blocking of IgE significantly reduced mast cell numbers in the colon and protected BALB/c mice from the onset of colitis.
Conclusions: T ogether, these data strongly suggest that IL-4-promoted $CD4^+$ T helper 2 cells producing IL-13 and IL-4-induced IgE production by B cells mediate oxazolone-induced colitis in concert.

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Epitope selection and protocol optimisation for peptide immunotherapy of type I diabetes in man using a humanised mouse model

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Administration of β cell antigen derived peptides is effective at preventing diabetes in animal models. However, identification of peptides recognised by auto-reactive T cells that have relevance to disease in human studies is difficult as auto-reactive cell frequencies are very low ($\leq$1 in 10^5) in peripheral blood. Furthermore, assessing whether T cells specific for a particular peptide are pathogenic is challenging in humans. We aim to develop an animal model for improvement of target peptide identification relevant to humans, confirmation of disease prevention and optimisation of peptide immunisation protocols. As MHC class II molecules are critical determinants of genetic susceptibility to human type I diabetes particularly the common haplotype HLA-DR4-DQ8, we are using a transgenic mouse model expressing these human HLA but lacking the endogenous mouse MHCII. These mice also express B7.1 (CD80) co-stimulatory molecules on the pancreatic β cells and are known as HLA-DQ8$^+$DR4$^+$mII$^-$/RIP-B7.1 or QRB7. Initial work shows on QRB7 primary immunisation, CD4$^+$ T cells proliferate and produce IFNγ in response to a pro-insulin peptide (C19-A3), previously tested in phase I immunotherapy trials. We used this response to evaluate protocols for *in vivo* induction of tolerance as well as for CD4$^+$ T cell line generation.

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CD200 inhibits memory Th1 cell function in acute myeloid leukaemia (AML)

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CD200 is a cell-surface glycoprotein that is normally expressed in tissues of the immune system, where its role is to protect immune privileged sites. We previously established CD200 to be frequently over-expressed and associated with poor AML patient outcome. In this study, we investigated the possibility that CD200 expression may mediate suppression of T-cell function in this disease. Using multi-parameter flow cytometry, we compared PMA/ionomycin stimulated CD8$^+$ T-cell cytotoxic potential (CD107a expression) and the frequency of intracellular TNFα, IL-2 and IFNγ producing CD4$^+$/CD8$^+$ memory T-cells between CD200^{hi} and CD200^{lo} patients. We demonstrated that both the magnitude of the CD8$^+$ memory cytotoxic T-cell response and the Th1 cytokine producing CD4$^+$ memory helper T-cells was significantly inhibited in CD200^{hi} AML patients ($P < 0.05$). Further, using ELISPOT assays to measure IFNg release we showed that the Th1 memory response to common viral antigens was significantly reduced by 75% in CD200^{hi} versus CD200^{lo} AML patients ($P < 0.05$). Recovery of IFNγ release in response to recall antigens was observed in CD4$^+$ memory T-cells incubated with a blocking antibody to CD200R. In conclusion, this study shows a correlation between T-cell dysfunction and expression of CD200 which suggests targeting this axis could be therapeutically beneficial for AML CD200^{hi} patients.

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A novel iron chelator reduces pro-inflammatory T cell responses and severity of disease in experimental arthritis

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Iron is essential to most living organisms and mammals have evolved strategies to withold iron from invading pathogens. One such strategy sequesters iron in immune antigen presenting cells (APCs) and leads to Anaemia of inflammation (AI) which is commonly associated with Rheumatoid Arthritis (RA). We hypothesised that iron overloaded APCs polarise T cells towards a pro-inflammatory T cell response. Therefore, studies were undertaken to investigate whether novel iron chelators could reduce inflammatory cytokine production which exacerbates RA symptoms. We have demonstrated in murine systems that antigen-specific T cell proliferation and production of the pro-inflammatory cytokines IL-17 and IFN-gamma are reduced by over 90% following treatment with a novel iron chelator SF34. Herein, we demonstrate in a human inflammatory model, where Toll Like Receptor 4 and CD3 T Cell Receptor agonists were added to isolated CD4$^+$ T cells and CD14$^+$ monocyte populations, that SF34 can reduce IL-17 by over 40%. This is further confirmed in a human Tetanus Toxoid model where antigen processing and presentation are required for antigen specific responses. Surprisingly, contrary to the original hypothesis the chelator primarily acts on the T cells rather than the APC. Furthermore in the experimental collagen induced arthritis model, injection of the chelator for a 2 week period during disease induction significantly reduces the clinical severity of disease. This study demonstrates the intrinsic ability of cellular iron to modulate inflammatory cytokine secretion and targeting this could potentially lead to a reduction in RA symptoms.

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BiP suppresses experimental arthritis by inducing regulatory cells

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Rheumatoid arthritis is a chronic, debilitating disease and is associated with articular inflammation and joint destruction. Immunisation with, and the adoptive transfer of Binding immunoglobulin protein (BiP)-reactive cells has been previously demonstrated to ameliorate collagen-induced arthritis (CIA). The aim of this study was to elucidate the mechanisms which underlie the immunomodulatory functions of BiP-responsive cells. Splenocytes and lymph node cells isolated from BiP-immunized mice were co-cultured in regulatory cell assays with type II collagen (CII)-reactive cells (the pathogenic cells in CIA). The *in vitro* regulatory potential of the BiP-reactive cells were assessed by their ability to suppress the recall responses of the CII-responder cells. In addition, phenotypic analysis via flow cytometry for regulatory T cell markers was performed. These studies revealed that BiP induces the development of antigen-specific Treg that are CD4+, CD25+, FoxP3−, CTLA-4+, PD-1^{high} that secrete the anti-inflammatory cytokines TGF-β (IL-6 is also secreted) and IL-10. Interestingly, intracellular CTLA-4 was also upregulated within the CD4+, FoxP3+ natural Treg population. Utilising neutralising antibodies in the regulatory cell assays it was shown that the secretion of IL-10 was one factor that contributed to suppressing proliferation and IFN-γ production. BiP is upregulated in the joints of mice with arthritis and this endogenous murine BiP separated from joints by SDS-PAGE and western blotting can stimulate T cells to secrete IL-10. It is proposed that BiP-immunisation induces cells that home to the inflamed joints, are activated by endogenous BiP, where they utilise several regulatory mechanisms that inhibit pathogenic responses and hence CIA.

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Polymeric human Fc-fusion proteins with modified effector functions

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The clinical success of IgG Fc-fusion bio-therapeutics has spurred the development of other Fc-fusion products for treating and/or vaccinating against a diverse range of diseases. We describe a method to modulate the *in vivo* function of Fc-fusion molecules by converting them into well-defined stable recombinant polymers using sequences from IgM. This strategy resulted in cylindrical hexameric structures revealed by tapping mode atomic force microscopy (AFM). Polymeric Fc-fusions were significantly less immunogenic than their dimeric or monomeric counterparts, a result partly owing to reduced ability to interact with critical classical Fcg-receptors (FcgRs) and the neonatal Fc-receptor (FcRn). However, in the absence of the fusion partner, polymeric IgG1-Fc molecules were capable of binding selectively to mouse and human FcgRs, with significantly increased affinity over the monomeric or dimeric Fc-fusions, likely owing to their increased valency, suggesting that these reagents may prove of immediate utility in the development of well-defined replacements for intravenous immunoglobulin (IVIG) therapy. Overall, these findings establish an effective IgG Fc-fusion based polymeric platform with which the therapeutic and vaccination applications of recombinant immune-complexes can now be explored.

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Effect of low-level treatment with an 80-Hz pulsed infrared diode laser on mast-cell numbers and degranulation in a rat model of third-degree burn

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Low-level laser therapy (LLLT) has been reported to be capable of changing mast cell numbers and degranulation in experimental burns in rats. We conducted a study of the influence of LLLT on mast cells in a rat model of third-degree burn. In this study we divided 48 rats equally into two groups of 24 rats each. Third-degree burns were inflicted at three different locations on each rat in each group. The first burn site on rats in group I was treated with 890-nm pulsed laser, 80 Hz, average power 1 mW, illuminated area 1 cm(2), 1 mW/cm(2), 856 s, 0.924 J/cm(2). The second burn site on both groups of rats was treated with 0.2% nitrofurazone cream. Mast cell numbers and degranulation at each burn site on each group of rats were then assessed at 4, 8, 13, and 20 days after the infliction of burns. Analysis of variance on day 4 showed that the total numbers of mast cells were significantly lower at the laser-treated burn sites than at other burn sites on both groups of rats. On day 8 the total numbers of mast cells were again significantly lower at the laser-treated burn sites than at other burn sites, and on day 13, the numbers of both types 1 and 2 mast cells were significantly lower at the laser-treated burn sites than at other burn sites. We conclude that LLLT can significantly decrease total numbers of mast cells during the proliferation and remodeling phases of healing in a rat model of third-degree burn.

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Anti-CD3 mAb induces CD8+FOXP3+ regulatory T cells from the PBMC of patients with rheumatoid arthritis

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Anti-CD3 monoclonal antibody (mAb) therapy for type 1 diabetes and organ transplantation has been linked with regulatory T cell (Treg) induction *in vivo*. Our aim was to determine whether anti-CD3 mAb could induce CD8+ Tregs, in the context of rheumatoid arthritis (RA), and their mechanism of induction. RA PBMC co-cultured *in vitro* with low-dose anti-CD3 mAb successfully induced CD8+FoxP3+ Tregs capable of suppressing the proliferation of SEB stimulated CD4+ T cells. CD8+FoxP3+ Tregs expressed high levels of TNFReceptor2, suggesting a role for TNF-α in their induction or maintenance. Blockade of TNF-α resulted in inhibition of CD8+FoxP3+ Treg induction by approximately half ($P < 0.001$). The cellular origin of TNF-α was ascertained by depletion of CD4+ T cells, B cells or monocytes from PBMC, revealing monocytes as the source. Inhibition of other cytokines produced by monocytes suggested that IL-6 and IL-1-β are required for maintenance of CD8+FoxP3+ Tregs. The requirement of a co-stimulatory signal to CD8+ T cells by monocytes was investigated by the blockade of CD80 and CD86, resulting in approximately 50% inhibition ($P < 0.001$) of induction by CD86 blockade only. This study demonstrates that two signals are provided by monocytes for the induction of CD8+FoxP3+ Tregs by anti-CD3 mAb: a co-stimulatory signal and TNF-α production.

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TNF-mediated macrophage activation in the target organ is critical for clinical manifestation of uveitis

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Clinically available anti-TNF biologics, which inhibit both soluble (sTNF) and transmembrane forms (tmTNF) of TNF, eliminating all TNF signalling, have successfully treated autoimmune diseases including uveitis. These have potentially serious side-effects such as reactivation of latent *Mycobacterium tuberculosis* and therefore, more specific inhibition of TNF signalling pathways may maintain clinical efficacy whilst reducing adverse effects. To determine the effects of specific pharmacological inhibition of sTNF on macrophage activation and migration, we used a mouse model of uveitis (experimental autoimmune uveoretinitis; EAU). We show that selective inhibition of sTNF is sufficient to suppress EAU by limiting inflammatory $CD11b^+$ macrophages and $CD4^+$ T cells migration into the eye. However, inhibition of both sTNF and tmTNF is required to inhibit IFNγ-induced CCR2, CD40, MHC class II and nitric oxide (NO), and signalling via tmTNF is sufficient to mediate tissue damage. In confirmation, intravitreal inhibition of sTNF alone did not suppress disease, and inflammatory cells that migrated into the eye, were activated, generating NO, and thus causing structural damage to the retina. In contrast, intravitreal inhibition of both sTNF and tmTNF suppressed macrophage activation and therefore disease. We conclude that sTNF is required for inflammatory cell infiltration into target tissue but at the tissue site, inhibition of both sTNF and tmTNF is required to inhibit macrophage activation and to protect from tissue damage.

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The effect of UVB light on T-regulatory cell number and immune function in humans

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Many autoimmune diseases (AIDs) increase in prevalence with distance from the equator, suggesting that sunlight may be protective. Moreover, numerous studies have now demonstrated that patients with AIDs have lower vitamin D levels, which is synthesised as a result of ultraviolet (UV) light exposure in skin. Vitamin D has an important role in regulating immune function, including that of T-regulatory cells. Patients with many AIDs exhibit impaired regulatory T-cell numbers and function. However, it has still not been established whether UV light exposure to skin affects Treg numbers or function in humans. We therefore investigated the effects of narrowband UVB light (311−313 nm) on vitamin D status and T-regulatory cell number in 22 patients due to undergo phototherapy for psoriasis. Peripheral blood was sampled for vitamin D2 and D3 levels, and immunophenotyping of T-regulatory cells at baseline, and after 2 and 4 weeks of phototherapy.

Phototherapy treatment was associated with an increase in vitamin D serum levels (mean change of 43.34 nM) as well as an increase in $CD25^+FoxP3^+$ T-regulatory cell numbers (0.52−1.46%). There were also associated decreases in proliferative (10 000 cpm decrease between visits 1 and 2) and cytokine [(IFN-γ (decrease of 1120 pg/ml between visits 1 and 2) and IL-10 (decrease of 554 pg/ml between visits 1 and 2)] responses to stimulation with anti-CD3/28.

UVB light stimulates vitamin D synthesis, which is associated with a positive effect on natural T-regulatory cell numbers. This may explain the latitude effects seen in some autoimmune diseases.

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Lactic acid bacteria and retinoic acid cooperatively increase the proportion of $CD103^+$ cells in a human dendritic-like cell line and human monocyte-derived dendritic cells

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Background: There are both tolerogenic $CD103^+$ and inflammatory $CD103^-$ dendritic cells (DCs) in the intestine. Disruption of the balance of these DC subsets leads to inflammatory bowel disease (IBD), so regulation of the balance will provide new therapeutic approaches. Retinoic acid (RA) is involved in the induction of $CD103^+$ DC *in vitro*. However, cooperative effects of RA and intestinal microbes, such as lactic acid bacteria (LAB) on $CD103^+/CD103^-$ DC balance are not reported.

Aim: Herein, we demonstrated the cooperative effect of LAB with RA on $CD103^+/CD103^-$ cell balance using human dendritic-like cell line KG-1 and monocyte-derived DCs (MoDCs).

Methods: KG-1 was treated with differentiation factors (PMA and ionomycin) into DCs in the presence or absence of RA and/or LAB. Three days after, the cells treated were collected and the proportion of $CD103^+$ cells was analyzed by flow cytometory. To examine the cooperative effect in normal DCs, human peripheral blood monocytes were treated with GM-CSF and IL-4 in the presence or absence of RA and/or LAB. Eight days after, the cells treated were analyzed as KG-1. We used LAB isolated from fermented food and human intestine.

Results: RA increased the proportion of $CD103^+$ cells in KG-1 as well as in MoDCs reported by Iliev. Some LAB significantly increased the proportion of $CD103^+$ cells cooperatively with RA. One of effective strains, *Lactobacillus brevis* KB290 showed the same cooperative effect in MoDCs.

Conclusion: These results suggested that some LAB cooperatively increase the $CD103^+/CD103^-$ DC ratio with RA and could improve symptoms of IBD.

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Investigating the role of the heat shock protein BiP on regulatory T cell frequency and function

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Heat shock proteins (HSP) have been described as pleiotropic proteins. Originally these proteins were known to act intracellularly but recent findings suggest that they can also be found in the circulation. Extracellular HSPs have been reported to modulate immune cell functions. T regulatory cells are central for immune suppression and peripheral tolerance. The aim of the present study is to investigate the role of soluble HSPs on T regulatory cell number and function. This aim is achieved by studying correlations between T reg and soluble HSPs (in plasma) and by performing *in vitro* T reg assays with or without HSP T reg pre-treatment. Data from 90 healthy donors showed no correlations between T reg frequency defined as CD4+CD25+CD127− population and soluble BiP in their plasma. However, BiP was strongly correlated with plasma levels of endogenous cytokines. Preliminary results show that BiP pre-treated T regs inhibited T responder proliferation to a similar extent compared to untreated T regs. However, treatment of T reg with BiP reduced IL-17, IFN-γ and TGF-β while IL-10 was increased compared to untreated T reg co-cultures. All preliminary data suggest that soluble BiP may alter T reg function by modulating cytokine profiles. We speculate that soluble BiP is a potent immunomodulator one mechanism being its ability to regulate T regulatory cell function.

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A retrospective descriptive case series examining vaccine responses to pneumococcal, meningococcal and *Haemophilus influenzae* vaccines in hyposplenic and coeliac patients

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Patients with hyposplenism face the risk of overwhelming fulminating sepsis due to a reduction in opsonisation of encapsulated bacteria and IgM titres; some vaccine hyporesponsiveness has also been reported. An unknown proportion of patients with coeliac disease are hyposplenic. This study, utilizing a retrospective descriptive case series was designed to show non-inferiority of antibody response between coeliac patients and surgical asplenics using a pneumococcal polyvalent vaccine (PPV), a pneumococcal conjugate vaccine (PCV), a meningococcal conjugate vaccine and a haemophilus influenza type B (HiB) vaccine. A secondary objective was comparing vaccine response between the PCV and PPV. The case series consisted of 81 coeliacs and 58 asplenic and compared age-stratified geometric titre concentrations (GMCs) and mean fold increases (MFIs) between these two groups.

PPV in coeliacs aged 16–54 responded to serotypes 1, 5, 14, 18C and 23F ($P < 0.05$), and over-55s to serotypes 5, 6B, 14, 18C and 19F. For coeliacs vaccinated with PCV, the 16–54s responded to 1, 5, 6, 3 and 7F, and the over-55s responded to 1, 5, 6B, 18C, 7F and 19A. The asplenic groups didn't respond. PPV23 response was better than PCV13 in the 55+ group. Responses to Meningococcal and HiB vaccine were statistically significant across all age ranges, with greater responses in the 16–54 coeliac group compared to asplenics.

Vaccination is recommended for coeliac patients, and is effective in some asplenic patients. PPV23 appears to be more effective in the over 55s. Antibiotic prophylaxis and suitable education are strongly recommended.

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Suppression of innate and T-cell mediated cytokine responses by cortisol and prevention by mifepristone

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Following stroke and other severe trauma, production of inflammatory cytokines by peripheral blood cells from patients is suppressed and this is associated with increased plasma cortisol. Prior to determining whether cortisol is responsible for the suppression, we sought to identify whether pathophysiological concentrations of cortisol were able to suppress cytokine production and if this could be prevented by the glucocorticoid antagonist mifepristone. Blood from eight healthy volunteers was diluted 1:1 in RPMI medium, containing the relevant stimulants and drugs. Innate responses were activated with 100 ng/ml LPS for 24h and T-cell responses with 5 μg/ml phytohaemagglutinin-leucoagglutinin (PHA-L) for 24 or 48 h at 37°, 5% CO_2. Cortisol at 10^{-6}–10^{-8} M and mifepristone at 10^{-5}–10^{-6} M, or control diluents, were added at culture initiation and supernatants were subsequently harvested by centrifugation. Supernatant IL-1ß, IL-4, IL-6, TNF-α and IFN-γ were measured by immunoassay. Induction of IL-1ß, IL-6 and TNF-α by LPS was inhibited in a dose-related fashion by cortisol added at 3×10^{-7}–10^{-6} M. After establishing that 24 h was the optimal time for induction of interleukin-4 (IL-4), IL-6 and interferon-γ (IFN-γ) by PHA-L, we found that induction of these cytokines was also inhibited by cortisol, when added at 10^{-7}–10^{-6} M. Inhibition of cytokine production by 10^{-6} M cortisol was effectively reversed by mifepristone at 10^{-5} M. In conclusion, we have shown that innate and T-cell-mediated responses can be inhibited by cortisol at concentrations achieved in plasma following stroke or similar trauma and that this inhibition may be prevented by the glucocorticoid antagonist mifepristone.

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Signalling through PD-1 inhibits mouse T cell proliferation

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Programmed Death-1 (PD-1, CD279) is an inhibitory receptor expressed on activated T cells and other activated immune cell subsets. PD-1 is thought to play a role in peripheral tolerance; thus directly targeting the PD-1 pathway may bring clinical benefit in autoimmune disease. The aim of this study was to investigate whether signalling through PD-1 would inhibit T cell proliferation. T cells were isolated from C57BL/6 splenocytes and cultured on immobilised anti-CD3 and PD-L1 fusion protein. Significant inhibition of proliferation and proinflammatory cytokine production was observed. This inhibition was reversed in the presence of an anti-ligand antibody. Subsequent experiments using T cells from a PD-1 knock-out mouse confirmed the inhibition was specific to signalling through PD-1. Assays were also established using T cells from a human PD-1 knock-in mouse. Signalling via immobilised anti-CD3 and an anti-human PD-1 antibody resulted in significant inhibition of T cell proliferation. This was reversed in the presence of recombinant PD-1. These studies support directly targeting PD-1 as a treatment for autoimmune disease.

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Regulatory T cells expressing granzyme B play a critical role in controlling lung inflammation during acute viral infection

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The inflammatory response to lung infections must be tightly regulated, enabling pathogen elimination while maintaining crucial gas exchange. Using recently described 'depletion of regulatory T cell' (DEREG) mice, we found that selective depletion of regulatory T cells (Tregs) during acute respiratory syncytial virus (RSV) infection enhanced viral clearance but increased weight loss, local cytokine and chemokine release, T cell activation and cellular influx into the lungs. Conversely, inflammation was decreased when Treg numbers and activity were boosted using IL-2 immune complexes. Unexpectedly, lung (but not draining lymph node) Tregs from RSV infected mice expressed granzyme B (GzmB) and bone marrow chimeric mice with selective loss of GzmB in the Treg compartment displayed markedly enhanced cellular infiltration into the lung after infection. A crucial role for GzmB expressing Tregs has not hitherto been described in the lung or during acute infections, but may explain the inability of children with perforin/GzmB defects to regulate immune responses to infection. The effects of RSV infection in mice with defective immune regulation closely parallel the observed effects of RSV in children with bronchiolitis, suggesting that the pathogenesis of bronchiolitis may involve an inability to regulate virus-induced inflammation.

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ST2 gene-deletion reveals Foxp3$^+$ regulatory T cells as a second mechanism of resistance to diabetes in BALB/c mice

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CD4$^+$Foxp3$^+$ regulatory T cells (Tregs) participate as one of the most important factor in limiting the autoimmune process. Therefore, we analyzed the ability of Tregs to suppress diabetogenesis in the multiple low dose of streptozoticin (MLD-STZ) experimental model of diabetes. Various dosages of cyclophosphamide (CY) were tested for effective depletion of Tregs. However, CY sensitive Tregs appear not to be involved in wild type BALB/c mice resistance to diabetes. Doses in the range of 20−175 mg/kg b.w. of CY did not enhance STZ induced diabetes in BALB/c mice, as evaluated by glycemia, glycosuria and infiltration of mononuclear cells in islets. We assume that Tregs control is the second line of deffence in this strain. Deletion of ST2 gene renders the relatively resistant mouse BALB/c strain susceptible to MLD-STZ induced diabetes. Therefore we tested whether and where Tregs control diabetes in ST2 knock-out (ST2KO) BALB/c mice. Treatment ST2KO mice with 50 mg/kg b.w. of CY enhanced glycemia, glycosuria and intrainsulitis which was accompanied with the increased levels of TNF-α and decreased IL-10 levels in the sera. CY treatment eliminated Tregs in pancreatic lymph nodes in BALB/c and ST2KO mice, while the markedly increased influx of Teffs in pancreata was observed only in ST2KO mice. ST2/Th2 signaling is a dominant mechanism of diabetes resistance, while ST2 gene-deletion revealed the role of Tregs as a second mechanism that controls the induction of diabetes mellitus in BALB/c mice. Tregs probably act in pancreatic lymph nodes rather than in the pancreatic tissue in susceptible ST2KO mice.

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TNFi therapy in rheumatoid arthritis converts proinflammatory T cells, including Th17 cells, to a regulatory state

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TNF-a inhibitor (TNFi) therapy is used with considerable success in the treatment of Rheumatoid Arthritis (RA). Here we investigated how TNFi therapy affects the expression of the inflammatory cytokine IL-17. Th17 cells (CD3+CD4+IL-17+ IFNγ) are enriched in the blood of patients with RA ($n = 40$) when compared to healthy controls ($n = 30$) [median (IQR) 0.5 (0.28−1.59)% versus 0.32 (0.21−0.54)%, $P = 0.005$]. When subdivided into treatment groups, Th17 cell frequency was further enriched in patients treated with TNFi compared to those on DMARD therapy (2.3 ± 1.7% versus 1.1 ± 0.8%, $P = 0.02$). This enrichment could not be explained by patient demographics or disease activity but instead appears to be a direct drug effect as *in vitro* addition of TNFi drugs (Infliximab, Adalimumab and Etanercept) significantly increased the percentage of IL-17-expressing CD4+ T cells. Critically, the Th17 cells generated in the presence of TNFi drugs displayed a novel regulatory phenotype with increased co-expression of IL-10. Significant IL-10 co-expression was also observed in Th1 and TNFα+ CD4+ T cells in the presence of TNFi. These data suggest that TNFi therapy has an additional benefit by converting pro-inflammatory T cells, including Th17 cells, to a regulatory phenotype.

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Immunostimulatory monoclonal antibodies combined with peptide vaccination provides potent immunotherapy in an aggressive murine neuroblastoma model

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Neuroblastoma is one of the commonest extra-cranial tumours of childhood. Over 50% of children present with metastatic disease and consequentially long term survival remains poor, despite intensive multi-modal therapies. Targeted immunotherapy is potentially a more specific and less toxic treatment than conventional therapies, with a number of tumour-associated antigens, including tyrosine hydroxylase and survivin, being identified as candidates for vaccination. Both these antigens are expressed in 80−100% of high-risk tumours but only minimally in normal tissue. Spontaneous anti-survivin T-cell responses have been reported in almost 90% of patients, but these responses clearly fail to control disease progression. Immunostimulatory monoclonal antibodies (mAb) targeting co-stimulatory molecules, such as 4-1BB and CTLA-4, can provide agonistic or counter-regulatory signals, offering a practical and potent means of boosting these weak endogenous responses to achieve therapeutic immunity.

Here we demonstrate the efficacy of immunostimulatory mAb in murine syngeneic neuroblastoma models, where treatment of established, weakly immunogenic tumour with mAb resulted in resolution of tumour, long-term survival and protection from tumour re-challenge. Survival was dependent upon the presence of tumour-associated antigen and was abolished after NK and CD8$^+$ T-cell depletion. In similar experiments, using a more aggressive tumour, survival was not observed with administration of immunostimulatory mAb alone. However long-term survival was achieved in 60% of mice when mAb was administration in conjunction with specific peptide vaccination. In addition, survival was associated with the generation of peptide-specific T-cell immunity. These data suggests the combination of antigen and co-stimulatory mAb may provide effective immunotherapy against neuroblastoma.

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Stroke-associated immune suppression is reversed by interleukin-1 receptor antagonist and is related to hypothalamic-pituitary-adrenal axis activity

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Infections are common following stroke and adversely affect outcome. We previously showed induction of peripheral blood cytokines in response to bacterial lipopolysaccharide was reduced in stroke patients. Cytokines importantly contribute to both stroke pathology and the response to infection. Since interleukin-1 receptor antagonist (IL-1Ra) is a candidate treatment for cerebral ischemia, we examined whether its administration to acute stroke patients affected cytokine induction in a phase II placebo-controlled trial. Blood samples were collected from patients prior to and following randomisation, and from stroke-free controls. *In vitro* induction of IL-1β, tumour necrosis factor (TNF)-a, IL-6, IL-8 and IL-10 by lipopolysaccharide was significantly reduced in patients at admission, when compared to controls. At 24 h, cytokine induction remained suppressed in the placebo group. In contrast, for patients treated with IL-1Ra, induction of TNF-a, IL-6 and IL-10 was similar to controls and IL-1β induction was significantly greater than in the placebo group. At 5–7 days IL-1β and TNF-a induction remained suppressed only in the placebo group ($P < 0.05$). Compared to controls, plasma cortisol concentrations were elevated in patients at admission prior to receiving either placebo or IV IL-1Ra. However, plasma cortisol concentrations at 24 h were substantially reduced in patients receiving IV IL-1Ra compared to placebo ($P < 0.05$). A significant inverse correlation was observed between plasma cortisol at admission and either TNF-a ($r = -0.71$, $P < 0.001$) or IL-1β induction ($r = -0.67$, $P < 0.001$) at admission. Reversal of suppressed innate cellular immune responsiveness and cortisol production by IL-1Ra supports a role for the hypothalamic-pituitary-adrenal axis in immune suppression following stroke.

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Serotonin reuptake inhibitors selectively decrease proliferation and viability of activated T-cells

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Serotonin reuptake inhibitors (SRIs) are widely prescribed drugs for the treatment of depression. Although SRIs are generally regarded as safe drugs with relatively few side effects, they may compromise the cellular immune response. High concentrations of SRIs have been shown to alter lymphocyte proliferation, behavior and viability, and therefore several reports propose SRIs as a possible therapy for autoimmune pathologies. However, if SRIs are considered for the treatment of immune-mediated disorders, they should preferably be able to selectively target unwanted activated T-cells without affecting resting T-cells. This study investigated the effect of six SRIs (paroxetine, fluoxetine, sertraline, fluvoxamine, citalopram and venlafaxine) on apoptosis of activated and resting T-cells by annexin V and propidium iodide staining. In addition, the effect of SRIs on the proliferation of activated T-cells was determined by CFSE staining. A pro-apoptotic effect was detected for the SRIs paroxetine, fluoxetine, sertraline, fluvoxamine and citalopram in activated T-cells ($n = 6$). The strongest effect was observed for paroxetine and sertraline, which significantly induced apoptosis in activated T-cells at 5 μM. The apoptotic effect of SRIs on resting T-cells was significantly lower. No apoptotic effect could be detected for venlafaxine. In addition, this study also showed that SRIs reduced T-cell proliferation ($n = 6$). For fluoxetine and sertraline, concentrations as low as 1 μM already significantly reduced T-cell proliferation. These results indicate that SRIs might be useful for selective targeting of activated T-cells, e.g. in the treatment of autoimmune pathologies.

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Investigation of D4Ply as a novel vaccine candidate against pneumococcal disease in humans

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Background: Pneumococcal infection causes significant morbidity and mortality worldwide. In excess of 1 million children die each year from this infection, mainly in developing countries. Currently available vaccines only have limited efficacy since they target a restricted number of serotypes. Pneumolysin (Ply) is a protein toxin that is released by virtually all serotypes. Ply has four domains, which together pore formation on cell membrane. The fourth domain (D4Ply) is important in cell binding. We aim to investigate the potential of D4Ply as a human vaccine.

Methods: A recombinant domain 4 Ply (D4Ply), which lacks toxicity was created and used to test its ability to activate antigen-presenting cells and T cells. Peripheral blood derived $CD14^+$ monocyte and monocyte cell line (THP-1) were used to analyse expression of co-stimulatory molecules (e.g. CD40, CD54, CD80 and CD86) and cytokine production after stimulation by D4Ply with flow-cytometry and ELISA. In addition, proliferation of peripheral blood mononuclear cells was examined using CFSE staining.

Results and conclusion: Preliminary results suggest that D4Ply can activate antigen presenting cells, evidenced by upregulation of co-stimulatory molecules (e.g. CD40, CD54, CD80 and CD86) and TLR2 and TLR4, as well cytokines TNF-α, IL-10 and IL-1β. In addition, D4Ply induced a proliferative response in $CD4^+$ T cells and production of cytokines including IL-17 and INF-γ. Further studies are underway to understand the mechanisms by which D4Ply induce the activation of these immune cells.

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Enriched FoxP3$^+$ T regulatory cells in renal cell carcinoma patients co-express Helios, indicating they could be derived from natural but not induced Tregs

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Background: Several studies reported the expansion of peripheral and tumour-infiltrating T regulatory cells (Tregs) in cancer patients, but the mechanism of Treg expansion is not evident. Conversion of conventional T cells into Tregs has been proposed as a potential mechanism; however, this evidence is supported by *in vitro* or mouse model studies with no data from *in vivo* or human studies to support its role in enriching peripheral and tumour-infiltrating Tregs. Recent work has shown that induced FoxP3$^+$ Tregs do not express Helios; an Ikaros family transcription factor. We analyzed peripheral blood samples from untreated renal cell carcinoma (RCC) patients, IL-2-treated RCC patients and tumour-infiltrating lymphocytes for the expression of FoxP3 and Helios.
Results: Our work shows that expanded peripheral FoxP3$^+$ Tregs in untreated RCC patients co-express Helios, which shows that they are derived from natural (nTregs) but not induced Tregs (iTregs). Interestingly, IL-2 administration results in expansion of FoxP3$^+$Helios$^+$ nTregs significantly more than FoxP3$^+$Helios$^-$ iTregs. Additionally, we report that the vast majority of FoxP3$^+$ Tregs in metastatic lesions co-express Helios. These expanded nTregs could be generated by the proliferation of pre-existing Tregs in unknown mechanism.
Conclusions: Our results show that the increased FoxP3$^+$ T regulatory cells in cancer patients co-express Helios, indicating that they could be derived from natural but not induced Tregs. This work may validate the potential targeting of Helios for reducing Treg numbers and/or activity in cancer patients.

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Differential effect of memory, naive and transitional B cells in autologous CD4$^+$ T cell proliferation, activation and cytokine production

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Background: Biomarker studies have identified a B-cell specific gene expression profile that is associated with immunological tolerance in kidney transplant recipients (Sagoo et.al. JCI 2010). Transitional B-cells have recently been shown to display anti-inflammatory properties, and consequently could potentially contribute to the maintenance of graft tolerance.
Hypothesis: Transitional B-cells would inhibit T-cell proliferation, activation and inflammatory cytokine expression.
Methods: Memory, naïve and transitional B cells were sorted and co-cultured with autologous CD4$^+$T cells. CD20, CD4, CD69, CD25, CD86 IFN-g, TNF-a and IL-17 expression were measured by flow cytometry. Also, IFN-g production was measured by ELISA. B cell subsets from PBMC samples from three groups of kidney transplant recipients (tolerant, stable, chronic rejector) and healthy controls were analysed by FACS.
Results: Transitional B cells were more prevalent in tolerant patients (10.8%) in comparison with stable patients (5.0%) or chronic rejectors (2.1%). Furthermore, in contrast to memory B-cells, transitional B cells were incapable of stimulating proliferation, activation or pro-inflammatory cytokine production by autologous CD4$^+$ T-cells. Notably, addition of CD14$^+$ monocytes to T- and B-cell co-cultures was capable of overcoming transitional B-cell inhibition.
Conclusions: Unlike memory B cells, transitional B cells are unable to induce activation of CD4$^+$T cells. This may be due to a difference in the expression of co-stimulatory molecules, impaired survival of transitional B cells in culture, or to changes in cytokine expression profile. None of the B cells subsets affected the interaction between CD4$^+$ and CD14$^+$ cells.
GAMBIT Study Consortium

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Study of the effect of CAMPATH on cord blood and peripheral blood cells

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CAMPATH is an anti-CD52 monoclonal antibody used as an immunosuppressive drug that effectively depletes T cells in the pre-transplant conditioning regimen.

CD52 expression on resting peripheral blood (PB) and cord blood (CB) cells was studied. For CB, the level of CD52 expression on B cells and T cells was comparable, natural killer (NK) cells expressed the lowest level of CD52, and CD52 was not expressed on CB stem cells. For PB, B cells expressed the highest CD52 expression, followed by T cells, NKT cells, and NK cells. Naïve T cells expressed higher levels of CD52 than memory T cells in PB and CB, and regulatory T (Treg) cells had the lowest CD52 density. CD52 expression was generally significantly higher on CB than in PB T cell subsets, except for Treg cells where CD52 expression was comparable in both.

A viability study was designed to study the potency of CAMPATH in inducing PB and CB immune cell death. Although different concentrations were used, CAMPATH induced apoptosis and necrosis in a comparable manner, having the same effects on both CB/PB T and B cells. No difference was shown for both CB/PB NK cells. Unlike PB Treg cells, CB Treg cells did not respond to CAMPATH. A maximum response towards CAMPATH treatment was observed approximately 24-h post administration for all cell types.

A better understanding of the impact of CAMPATH on immune cells would be useful to contribute to the future clinical application of the drug in order to achieve optimal efficacy.

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Helminth-derived immunomodulator AvCystatin blocks Th2 driven lung eosinophillia in mice infected with respiratory syncytial virus

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Respiratory syncytial virus (RSV) is the major world-wide cause of viral bronchiolitis, which is the commonest cause of acute lower respiratory tract illness in infancy. Pathology seems to be mainly dependant on excessive immune responses (immunopathology).

Parasitic worms have evolved potent modulators of immune responses in order to evade or block immune responses directed against them and persist in their hosts, thereby potentially blocking other host immune reactions as well. For example, the helminth-derived immunomodulator AvCystatin has successfully been used to attenuate murine allergic airway inflammation.

We therefore investigated the effect of recombinant AvCystatin in RSV-induced eosinophillic airway inflammation in BALB/c mice sensitized with vaccinia virus expressing the RSV G-protein (vvG), which causes Th2-mediated enhanced inflammation. AvCystatin was shown to provide immunomodulation specific to the airway inflammation, as viral load was not enhanced and viral persistence not prolonged. Application of the immunomodulator was then tested in different application settings (intranasal versus intraperitoneal) and timings (preventative versus prechallenge). Treatment of mice with AvCystatin completely ablated eosinophil influx into the airway, reduced excessive weight loss and diminished Th2 cytokine (IL-4, IL-13) and chemokine (RANTES, Eotaxin) production. In association with these changes, AvCystatin increased numbers of $CD4^+$ $IL\text{-}10^+$ T cells in lung and airways of RSV challenged animals, indicating an induction of protective Tr1 and Treg cells depending on timing and application mode.

Natural immune modulators from helminths employ different mechanisms and cells to evade or alter host immune reactions and are potent candidates for treatment of diseases caused by enhanced immune responses.

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Contortion of the oncogenic protein LMO2 structure *in vivo* by a single VH domain antibody causes functional inhibition

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LMO2 is a LIM-only protein that forms part of a multi-protein transcription factor complex, together with LDB1, E47 and TAL1, among other proteins. LMO2 was originally discovered through chromosomal translocations with T cell receptor delta or beta chain genes in patients with T-cell acute lymphoblastic leukaemia. Expression of LMO2 is also a prognostic factor in diffuse large B cell lymphoma. We have recently developed a single VH domain antibody as a drug surrogate that binds Lmo2 inside cells and inhibits tumourigenesis in an Lmo2-dependent leukaemia model. We have solved the crystal structure of LMO2 in complex with the anti-LMO2 VH to determine the mechanism of functional inhibition by the VH. Comparing the structure of LMO2 in complex with either the anti-LMO2 VH or with LDB1 shows a significant conformational difference, specifically in the relative positioning and angle between the two LIM domains. These findings suggest a model in which initially synthesized LMO2 protein is intrinsically disordered and binding to a partner protein confers structure that dictates subsequent protein complex formation. The effect of the anti-LMO2 VH is to contort LMO2 into a conformation that interferes with the binding of natural partners, thereby preventing formation of functional transcription factor complexes. The anti-LMO2 VH is a starting point for a new type of therapeutic approach for the treatment of LMO2-dependent leukaemia/lymphoma.

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Development and assessment of live *Lactococcus lactis* -based mucosal vaccination as a immunoprophylactic strategy against *Leishmania infantum*

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Leishmaniasis is a parasitic disease affecting more than 12 million people worldwide, and constitutes a major public health problem particularly in poorer countries. There is also a recognised risk that the disease may become more prevalent in Europe due to global warming and migration. Treatment is prolonged and expensive and the development of a vaccine is a priority.

Dietary lactic acid bacteria have received considerable interest as mucosal vaccine delivery vehicles and have demonstrated potential to promote T helper 1 or mixed T helper cellular responses to expressed or co-administered antigen, considered advantageous in vaccination strategies targeting Leishmaniasis. With the aim to develop a new imunoprophylactic strategy against Leishmaniasis, we bioengineered the non-colonizing, non-pathogenic Gram-positive bacterium *Lactococcus lactis* to express vaccine candidate antigens (presented as intracellular antigen or anchored on the bacterial surface) from Leishmania infantum. When administered as live mucosal vaccines, we were able to confirm that these prototypes can elicit antigen-specific humoral and cellular responses in a Balb/c mouse model of visceral leishmaniasis (VL). We are currently assessing the efficacy of these vaccines against parasitic challenge. If successful, this program of research has potential to create a new path to preventing VL disease and other leishmaniases that is both inexpensive and amenable to large-scale vaccination programmes in populations who are at risk. There may also be scope to expand this approach to target other neglected diseases.

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Structures of enhanced affinity TCR reveal new insights in cancer recognition and high-affinity TCR specificity

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Malignant melanoma is the most aggressive and deadly form of skin cancer. It is responsible for 75% of all skin cancer-related deaths worldwide and the global incidence is rising. T-cells direct immune responses against pathogens and cancer through a specific interaction between the T-cell receptor (TCR) (expressed on the T-cell surface) and peptide-major histocompatibility complex (pMHC) molecules (expressed on the target cell surface). However, TCRs have a very low affinity for cancer antigens. In order to address this issue, we have developed engineered soluble TCRs that exhibit over a million-fold enhancement in affinity for tumour antigens.

We analysed the specificity of a high-affinity TCR toward melanoma by introducing a number of mutations into the melanoma antigen and used Surface Plasmon Resonance (SPR) to show that this TCR is exquisitely specific for the melanoma antigen and may be useful for therapies directed against skin cancer. We also determined the atomic structures of this TCR complexed to a melanoma antigen and two mutated melanoma antigens. This showed that the 30 000-fold reduction in the binding affinity is caused by some really subtle changes in the contacts between the TCR and the cancer peptide.

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Activation of cord blood natural killer cells

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Natural Killer (NK) cells are components of the innate immune system involved in killing viral infected cells and stressed cells. They can become activated upon encountering non-self major histocompatibility complex molecules. Cord blood (CB) has been recently used as an alternative source of hematopoietic stem cells for hematological malignancies treatment due to its off-the-shelf availability, tolerance of up to two human leucocyte antigen mismatches, reduced incidence and severity of graft versus host disease (GvHD), and preservation of the graft versus leukemia (GvL) effect. NK cells are prominent in CB constituting up to 30% of total mononuclear cells and their reconstitution occurs early after transplantation. *In-vivo*, NK cells traffic from the periphery in response to inflammatory signal where they acquire their cytolytic function. Cytokines released from dendritic cells and T lymphocytes promote NK cell activation and γ-IFN production. Here we propose that CB NK cells are responsive to cytokine treatment and become functional. A comparative study between cytokine conditioned peripheral blood (PB) NK and CB NK cells have been carried out. CB or PB NK cells were co-cultured with IL-2, IL-12, IL-15, and IL-18. Our results showed that CB NK cells are responsive to cytokines conditioning *ex-vivo*. This has been confirmed by up-regulation of NK cell triggering receptors, adhesion molecules, and lymph node homing receptors. Hence, cytokine conditioned CB NK cells could be used for therapeutic purposes in clinical settings to kill host's dendritic cells accounted for initiating GvHD and kill leukemia cells preserving the GvL effect.

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Post-rituximab panhypogammaglobulinaemia requiring intravenous immunoglobulin replacement therapy (IVIG)

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Background: Rituximab is a chimeric monoclonal antibody against CD20 used in the treatment of B-cell lymphomas and autoimmune conditions. Transient peripheral B cell depletion is expected following rituximab therapy. Although initial clinical trials did not show significant hypo-gammaglobulinaemia, case reports describing this have been appearing in the literature.
Methods: We present six patients previously treated with rituximab that developed symptomatic hypogammaglobulinaemia requiring IVIG.
Results: Five patients were treated with rituximab as well as combination chemotherapy for non-Hodgkin's lymphoma (follicular $n = 4$, marginal zone $n = 1$) and one patient for autoimmune haemolytic anaemia. Mean age was 48.7 years (range 24–61). Length of rituximab treatment ranged from over 3 years ($n = 3$) to <2 years ($n = 3$). Five of the patients presented with recurrent infections despite prophylactic antibiotics. All were found to have panhypogammaglobulinaemia and reduced or absent B cells. Haemophilus Influenzae B, tetanus and Pneumococcal total and serotype-specific antibody levels were all reduced. All patients failed to mount an immune response post-vaccination. The mean interval from the last dose of rituximab and need for IVIG was 23.7 ± 13.5 months (range 7–48 months).
Conclusion: Clinicians should be aware of the potential of rituximab to cause clinically significant hypogammaglobulinaemia. This may present even years after the last course and especially in patients receiving prolonged courses and should be actively looked for during follow-up.

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Circulating anti-CCP antibodies and cytokines as biomarkers of response to methotrexate in rheumatoid arthritis patients

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Circulating cytokines, as well as antibodies to cyclic citrullinated peptides (aCCP), predict both the future development and severity of rheumatoid arthritis (RA). However, little is known about the effects of chemotherapy on these biomarkers of disease and disease activity. In the current study, aCCP and a range of circulating cytokines/chemokines/growth factors representative of the Th1/Th2/macrophage/fibroblast axis were measured prior to and 6 months post-administration of methotrexate to a group of predominantly female African patients with RA ($n = 129$, 105 seropositive for aCCP), all of whom were disease modifying anti-rheumatic drug (DMARD)-naïve. Cytokines and aCCP in serum specimens were measured using multiplex bead array technology and immunofluorometric procedures respectively. Methotrexate therapy was associated with significant ($P < 0.00000$) improvements in the simplified disease activity index, as well as with significant decreases in the circulating concentrations of aCCP, with pre-and post-treatment values of 657 ± 635 and 372 ± 388 units/ml ($P < 0.00000$) respectively. In the case of the cytokines, significant decreases were noted for IL-7, IL-8 and VEGF in particular ($P = 0.0005$ to <0.0000), and to a lesser extent for IL-4, IL-6 and G-CSF ($P = 0.0.02–0.007$), while those of IL-1β, IL1-Ra, IL-2, IL-10, IL-12, IL-17, GM-CSF, IFN-γ, TNF, CCL2 and CCL4 showed no significant changes ($P = 0.07–0.9$). These findings demonstrate that aCCP, IL-7, IL-8 and VEGF are potentially useful biomarkers of responses to methotrexate therapy in RA.

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Generation of natural killer cells from cord blood stem cells, characterization and application for immunotherapy

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NK cells are lymphocytes from the innate immunity able to kill tumor and virus infected cells without prior sensitization. They are important effectors of the immune response during transplantation, have a role in reproduction, and in mucosal immunity. Cord blood transplantation (CBT) is increasingly used as a source of stem cells for the treatment of hematological malignancies in adults. Advantages are increased access to the allo-therapy due to a decreased HLA restriction and off-the-shelf availability. After CBT, severity of graft versus host disease (GvHD) is reduced, and the Graft versus Leukemia (GvL) effect is preserved. We think that cord blood (CB) NK cells are key effectors of the immune response after CBT. Currently, more than 200 clinical trials have explored the possibility to use NK cells in immunotherapy for different types of cancer including: leukemia, melanoma, renal cell carcinoma, and breast, pancreas, lungs and head/neck carcinomas. The adoptive NK cell therapy relies on the use of large amount of NK cells that are cytotoxic and yet not exhausted. Here we compare the potential capacity of fresh CB stem cells and frozen CB and peripheral blood mobilized stem cells under different culture conditions to give rise to NK cells that could be used for immunotherapy. Furthermore, we have investigated the phenotypic and functional characteristics of the produced NK cells. This study will help to understand better the therapeutic potential of NK cells newly generated from cord blood.

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***In vivo* SPECT reporter gene imaging of regulatory T cells**

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Regulatory T cells (Tregs) were identified several years ago and are key in controlling autoimmune diseases and limiting immune responses to foreign antigens. Imaging of the human sodium/iodide symporter via Single Photon Emission Computed Tomography (SPECT) has been used to image various cell types *in vivo*. It has several advantages over other imaging techniques including high sensitivity, it allows non-invasive whole body studies of viable cell migration and localisation of cells over time and lastly it may offer the possibility to be translated to the clinic. This study addresses whether SPECT/CT imaging can be used to visualise the migratory pattern of Tregs *in vivo*. Treg lines derived from $CD4^+CD25^+FoxP3^+$ cells were retrovirally transduced with a construct encoding for the human Sodium Iodide Symporter (NIS) and the fluorescent protein mCherry and stimulated with autologous DCs. NIS expressing self-specific Tregs were specifically radiolabelled *in vitro* with Technetium-99m pertechnetate ($^{99m}TcO_4^-$) and exposure of these cells to radioactivity did not affect cell viability, phenotype or function. In addition adoptively transferred Treg-NIS cells were imaged *in vivo* in C57BL/6 (BL/6) mice by SPECT/CT using $^{99m}TcO_4^-$. After 24 h NIS expressing Tregs were observed in the spleen and their localisation was further confirmed by organ biodistribution studies and flow cytometry analysis. The data presented here suggests that SPECT/CT imaging can be utilised in preclinical imaging studies of adoptively transferred Tregs without affecting Treg function and viability thereby allowing longitudinal studies within disease models.

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The bone marrow adherent stem cell product Multistem® suppresses *in vitro* T cell responses relevant to allogeneic islet transplant rejection

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Type 1 Diabetes is a chronic autoimmune disorder characterized by the destruction of insulin-secreting β cells in the islet of Langerhans in the pancreas. This process, which is driven by β-cell-associated islet auto-antigen-specific autoreactive T cells, ultimately leads to the loss of blood glucose homeostasis. One treatment option available for a selective group of T1D patients is allogeneic islet transplantation, whereby donated islets can provide recipients with a means of restoring endogenous insulin production. However, within 90% of recipients graft function typically deteriorates by 5 years following the emergence of a complex anti-graft immune response. This response has been associated with the appearance of allo-specific T cells, a resurgence of auto-antigen specific T cells and the γ-chain cytokine-dependent homeostatic expansion of autoreactive memory T cells.

The recently described immunodulatory potential of adherent stem cells advocates the therapeutic application of these cells in clinical indications with tractable immunopathology. We have performed a comprehensive *in vitro* evaluation of the suitability of the bone marrow derived adherent stem cell product Multistem®, developed by Athersys™ Inc, to promote the engraftment and/or rescue of allogeneic islet transplants. We find that Multistem® suppresses the proliferation and effector function of allo-specific T cells as well as reactivated pre-existing Ag-specific memory T cells. Furthermore, we have discerned that Multistem® is capable of inhibiting γ-chain cytokine-dependent homeostatic T cell proliferation. Taken collectively, our study suggests that Multistem® exhibits several desirable properties *in vitro* that may support application as a clinical cell-therapy for use in human allogeneic islet transplantation.

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False positive hepatitis B serology following IVIG therapy: forgotten but not gone!

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Anti-B cell therapy with Rituximab is increasingly important for management of autoimmune disease. A key risk of this drug is the reactivation of latent hepatitis B and consequently patients are screened prior to treatment for evidence of past infection.

We recently identified four patients with pemphigus who were considered for Rituximab therapy in whom screening revealed anti-HBc IgG antibodies in the absence of anti-HBc IgM and HBsAg, consistent with past hepatitis B infection. Following specialist advice two patients had been given lamivudine prophylaxis. However, subsequent chart review revealed that all four patients had recently received IVIG for treatment of their disease and the possibility that anti-HBc had been passively acquired from the IVIG was considered. Consistent with this, anti-HBc was no longer detectable in patient sera 4–6 weeks later. Analysis of archived sera taken prior to IVIG did not demonstrate anti-HBc antibodies. Moreover, subsequent testing of several batches of Vigam IVIG in our clinic showed that 80% were anti-HBc positive.

Discussion with manufacturers of the Vigam brand of IVIG revealed that IVIG is currently sourced from donors who are screened for HBsAg and then pooled donations undergo HBV DNA screening. Consequently, contributions from individuals with cleared past HBV infection are not screened out. Our observation represents an important lesson as patients may receive IVIG for treatment of many immunologically mediated diseases. Awareness of passive anti-HbC acquisition will prevent future inappropriate exposure of patients to antiviral medication and allay anxiety consequent upon an incorrect diagnosis of past hepatitis B infection.

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Further insights into Salmonella mediated protection from type 1 diabetes in the NOD mouse

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Type 1 diabetes is a prevalent autoimmune disorder where a dysregulated T_H1 (T cell) response leads to the destruction of the insulin secreting β-cells in the pancreas, which in turn results in impaired glucose homeostasis. Intensive research has been carried out in an attempt to clarify the complex genetic component of T1D development. However, it is becoming evident that susceptibility to T1D is not totally dependent on genetic factors alone and the remaining risk factors must arise from the environment. In the NOD mouse model of T1D, a single infection with *Salmonella typhimurium* is sufficient to prevent the onset of diabetes, and there is some evidence that changes within the DC compartment play a crucial role in this protective effect. In this work, transcriptome analysis of DCs identified a distinct Salmonella-induced signature (heavily influenced by IFN-γ) in which the inhibitory receptor PD-L1 was up-regulated. Antibody mediated *in vivo* blockade of PD-L1 was found to ablate the protective function of Salmonella infection. Interestingly we find that, contrary to other infection-mediated models of T1D protection, there was no expansion of $Foxp3^+$ Tregs, however there was a significant reduction in the proliferative capacity of splenic $CD4^+$ $CD25^-$ T-cells. These data provide evidence for a novel regulatory DC phenotype proficient at controlling autoreactive T cells for an extended duration in the NOD mouse model of diabetes.

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Differential production of IL-17 expression in peripheral blood cultures of steroid refractory versus steroid sensitive asthmatics

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Background: Approximately 10% of the 5.4 million asthma patients in the UK cannot control their asthma with steroids, representing those most at risk. The cytokine IL-17 plays a protective role in host defense to respiratory infections, promoting airway neutrophilia. However evidence from a mouse model of asthma suggests its importance in steroid refractory airway disease.

Methods: A patient cohort currently enrolled in a clinical trial to test the therapeutic potential of active vitamin D3 (1,25[OH]D3) in steroid refractory asthma (SR) allowed us to assess IL-17 synthesis in PBMC cultures from SR and steroid sensitive (SS) patients.

Results: Higher levels of IL-17 synthesis occurred in cultures of polyclonally-activated PBMC from SR as compared to SS patients. The glucocorticoid dexamethasone failed to inhibit IL-17 production in SR cultures. In contrast dexamethasone enhanced IL-17 synthesis in SS PBMC, although IL-17 levels remained much lower than those measured in SR patient cultures. 1,25[OH]D3 potently inhibited IL-17 synthesis in all SR and SS cultures.

Conclusions: Our data provide further evidence for a role of IL-17 in steroid refractory asthma, highlighting a distinct pattern of IL-17 production in SR versus SS asthmatics. It seems plausible that whilst moderate amounts of IL-17 maybe beneficial for host defense, excessive production may contribute to autoimmune pathology. Although steroids fail to inhibit IL-17 in SR asthma patients, vitamin D potently inhibits IL-17 synthesis. A high prevalence of vitamin D insufficiency exists in severe asthma, warranting further investigation of the therapeutic role of vitamin D in asthma.

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Essential cross-linking role for FcgRIIB in the *in vivo* activity of anti-CD40 monoclonal antibody

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The optimal Fcg receptor (FcgR) binding profile of therapeutic anti-cancer mAb depends upon their mechanism of action. Thus, while agents like rituximab require interaction with activatory FcgR to stimulate effector cell mediated cancer cell clearance, we recently showed that interaction with the inhibitory FcgRIIB is critical for immunostimulatory anti-CD40. Experiments with chimeric mouse IgG1 (m1) and IgG2a (m2a) versions of the rat anti-mouse CD40 mAb 3/23 demonstrate that differences in affinity for FcgRIIB dictate that 3/23 m1, but not m2a, can stimulate immunity against OVA and moreover provide therapy for lymphoma in mouse models. Using *in vitro* activation of B cells as a measure of anti-CD40 activity, we demonstrate that the role of FcgRIIB is to provide mAb cross-linking and that intracellular signalling through FcgRIIB is not required. Activatory FcgR could also cross-link 3/23 m1 and/or m2a *in vitro* dependent upon their Fc binding affinities, suggesting the critical role for FcgRIIB *in vivo* reflects its bioavailability. Importantly m1, but not m2a or human IgG1 versions of the anti-hCD40 mAb LOB7.4 could activate hCD40 transgenic mouse B cells *in vitro*, whereas, consistent with their universal low affinity for hFcgRIIB, none could activate human B cells unless an appropriate cross-linking FcgR was supplied. Thus, our studies demonstrate that FcgR-mediated cross-linking is required for anti-CD40 activity and that FcgRIIB fulfils this role *in vivo*. It is tempting to speculate that the optimal FcgR binding profile of a therapeutic mAb will reflect both its mechanism and site of action.

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Targeting therapeutics to arthritic joints

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Background: The aim of our study is to target anti-inflammatory proteins to arthritic joints, in order to improve efficacy and reduce side-effects of current therapies.
Methods: We chose type II collagen (CII) as a target as it is uniquely present in cartilage. In the arthritic joint, CII is damaged by reactive oxidant species (ROS) generated in the inflammation process. We used ROS-modified CII (ROS-CII) to select a single chain fragment variable (scFv) specific to ROS-CII.

In order to target therapeutic proteins to the inflamed joints, we fused anti-ROS-CII scFv to anti-inflammatory proteins via MMP-1 cleavage site linker.
Results: We were able to demonstrate binding of anti-ROS-CII scFv 1-11E to damaged cartilage from rheumatoid arthritis (RA) and osteoarthritis (OA) but not to intact cartilage.

Accordingly, imaging studies have shown that fluorescently labelled 1-11E localises specifically to inflamed joints in arthritic mice.

1-11E fused to mTNFR2-Fc is cleaved at the linker site by MMP-1, and is biologically active *in vitro*. Moreover, we show that by fusing mTNFR2-Fc to 1-11E, the therapeutic efficacy in arthritic mice is enhanced.
Conclusion: We have a proof of principle that a therapeutic targeted by anti-ROS-CII to the joints has augmented anti-inflammatory properties. Future work will involve optimising the fusion proteins for development towards the clinic, including targeting alternative therapeutic molecules.

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Redistribution of CD20 and Fcγ receptor IIb (CD32b) into lipid rafts is important for internalisation of CD20 upon ligation with type I anti-CD20 mAbs

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We recently observed that type I, but not type II anti-CD20 mAbs mediate internalisation of CD20 from the surface of both normal and malignant B cells via a CD32b-dependent mechanism, the molecular basis of which remains unclear. The key defining difference between type I and type II anti-CD20 mAbs is the ability of type I mAbs to mediate the redistribution of CD20 into lipid rafts. Like CD20, CD32b is constitutively located outside of lipid rafts, but redistributes into raft regions upon crosslinking with specific mAb.

To determine if raft redistribution is important for CD20 internalisation, we transfected a human $CD20^{-ve}$ and $CD32b^{-ve}$ B cell line with either wild type CD20 or a mutant form that does not redistribute into rafts upon ligation (WN CD20 lacking residues 216–226). Using a flow cytometry based internalisation assay we observed that only the wild type form of CD20 was internalised upon ligation with type I anti-CD20 mAbs. Co-transfection of WN mutant CD20-expressing cells with CD32b restored the ability of type I anti-CD20 mAbs to mediate internalisation of WN CD20. Interestingly, this corresponded with an increased redistribution of WN CD20 into lipid rafts.

In support of these findings, $CD32b^{-ve}$ Ramos cells transfected with the I232T variant of CD32b, which has a reduced ability to redistribute into lipid rafts, also showed a decreased capacity to mediate internalisation of CD20 than cells containing wild type CD32b. These results suggest that raft redistribution of both CD20 and CD32b is important for internalisation of type I anti-CD20:CD20 complexes.

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Activation of dendritic cell subsets with Toll-like-receptor agonists for immunotherapy in ovarian carcinoma

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Dendritic cells (DC) have the potential to induce a tumour-specific adaptive immune response. Their ability to induce differentiation of naïve lymphocytes into effector cells in lymphoid tissues is dependent upon their activation. Malignant tumours can create an immunosuppressive environment in which DC are prevented from activation and the initiation of an anti-tumour immune response is hindered. The aim of our study is to investigate the effect of ovarian carcinoma (OC)-associated ascites fluid (AF) on DC activation by various Toll-like-receptor (TLR) agonists *in vitro*. Our results show that AF reduces the up-regulation of the co-stimulatory molecule CD86 and partially inhibits the production of IL-6, IL-12 and TNF-α in TLR-activated monocyte-derived DC from healthy volunteers *in vitro*. We have identified IL-10 as the pivotal component in AF jeopardizing DC activation, and we are in the process of examining the T-cell stimulatory capacity of such immune-compromised DC.

Future work is aimed at characterising the functionality of different DC subsets present in AF of patients suffering from the disease. We would like to explore whether by inducing TLR-mediated activation, OC-associated DC acquire immunogenic function and are capable of inducing anti-tumour immune responses. A thorough examination of these questions can provide substantial insight into the establishment of DC-based vaccines and concepts of immunotherapy in OC.

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Context-dependent regulation of T cell responses by CTLA-4

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Manipulating the CD28/CTLA-4 pathway is important to several immune-modulatory approaches in autoimmunity and cancer. Whilst CTLA-4 is a critical regulator of T cell responses, the immunological settings whereby it effectively restricts responses are not well defined. We tested a variety of conditions to establish where CTLA-4 was reproducibly inhibitory. During activation of resting human $CD4^+$ $CD25^-$ T cells we observed extensive T cell proliferation, in spite of robust CTLA-4 expression in all dividing T cells. These responses were both ligand (CD80/CD86) and CD28-dependent. However, blocking CTLA-4 antibodies had no impact on these responses, indicating the control by CTLA-4 is not ubiquitous or simply related to its expression. In contrast, in settings where CTLA-4 was present on 3rd party 'regulators', inhibition of responses was observed in a manner dependent on CTLA-4 expression and influenced by the antigen presenting cells in the culture. Moreover, the degree of suppression correlated with the level of downregulation of CD80 and CD86 from the antigen-presenting cell and acquisition by the $CTLA\text{-}4^+$ cell. Our data show that the inhibitory function of CTLA-4 is dependent on the level of costimulation available and is most effective as a T cell-extrinsic regulator of the APC. These results are compatible with trans-endocytosis [1] as a significant, robust and predictable mechanism of CTLA-4 function.

Reference:

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IMCmage1: a soluble TCR-anti-CD3 bi-functional reagent for redirected tumour cell killing

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Tumour associated antigen (TAA)-derived epitopes presented on human cancer cells in the context of HLA class-I molecules provide a marker for targeting by cytotoxic T cells. However, these cells often fail to clear tumours due to the low number of TAA epitopes on cancer cells coupled with the inherent low affinity of T cell Receptors (TCRs) for the target epitope. To overcome these issues we have made soluble versions of the native membrane-bound TCR molecules and affinity matured them using phage-display. We have generated bi-specific protein therapeutics, termed ImmTACs (immunne mobilising mTCR against cancer), to re-direct the immune system to target and destroy tumour cells.

IMCmage1 comprises a soluble, high-affinity TCR specific for the HLA-A1 presented peptide $MAGE_{168-175}$, fused to an anti-CD3 scFv domain. The TCR portion recognises TAA derived epitopes presented on cancer cells and the anti-CD3 moiety recruits and activates local T cells. We have demonstrated that IMCmage1 can effectively re-direct T cells to kill MAGE positive melanoma cells whilst leaving MAGE negative cells intact. No off-target activity was observed when IMCmage1 was tested against a panel of primary human cell lines.

A Phase I clinical trail using IMCmage1 (in multiple myeloma) is planned to commence in 2012.

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The rituximab-induced internalisation of CD20 on B-cells is independent of CD32B (FcγRIIB) signalling

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The anti-CD20 mAb, rituximab, has improved treatment outcomes in B-cell malignancies. However, several B-cell lymphomas either do not respond to rituximab or develop resistance. Internalisation of rituximab may be partly responsible for this resistance. We recently showed that the level of the inhibitory Fc receptor (CD32B) at the cell surface controls the rate of rituximab internalisation. However, the precise mechanism involved has not been elucidated. Different isoforms of CD32B exist (B1 and B2), only one of which (B2) has previously been associated with an ability to internalise. The B1 form in contrast contains an additional intracellular region that makes it more resistant to internalisation.

Therefore, we investigated the role of the two different CD32B isoforms (B1 and B2) and the associated intracellular tail on rituximab internalisation. CD32B1 and CD32B2 isoforms were stably transfected into $CD32B^{-ve}$ Ramos lymphoma cells, and flow cytometry was then used to determine the relative rates of rituximab internalisation. Additionally, we generated mutant versions of the CD32B receptors, including those lacking the entire cytoplasmic domain to assess the importance of intracellular signalling. The rate of rituximab internalisation in all of these studies was dependent on relative expression of CD32B and the CD20:CD32B ratio at the cell surface rather than any specific activity imparted by the CD32 intracellular domain. These studies suggest that the intracellular part of CD32B is redundant for rituximab-induced CD20 internalisation and imply that internalisation is augmented by CD32B simply through its ability to bind the Fc region of rituximab at the cell surface.

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Effect of biological response modifiers on respiratory burst response of murine macrophages *in vitro*

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Macrophages are the first line of defense and constitute important participant in the bi-directional interaction between innate and specific immunity. Also macrophages are amenable to activation by biological response modifiers (BRM) of different origin. It has been found that certain BRMs impart their function with a distinct duality. They are capable of acting as immunopotentiators. *Rasayans* are immunomodulatory herbal drug preparations described in *Ayurvedic* system of medicine, which exhibit a number of therapeutic properties. It is thought that the mechanisms involved in these effects are due to the modulation of innate immunity and more specifically, macrophage function. This led us to investigate the effect of *Guduchi* (*Tinospora cordifolia*) and LPS as a positive indicator on a macrophage cell line J774A. Supernatants collected from J774A cells treated with Guduchi, and LPS showed enhanced production of hydrogen peroxide, superoxide and TNF-α levels. It is suggested that increased production of these products represent activated state of macrophages. These activated macrophages could kill the tumor cells via cytolysis mediated by the release of the secretary products like H_2O_2, O_2^- and TNF-α.

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Peptide-based immunotherapy significantly reduces the severity of disease in an adoptive transfer model of ovalbumin-induced allergic airways inflammation

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Peptide-based immunotherapy (PIT) holds substantial therapeutic potential for the treatment of allergic disease. PIT utilises short allergen-derived immunodominant peptides to induce T cell tolerance. There is, however, a need for further understanding of the immunological mechanisms involved in successful PIT.

We have established a murine model of allergic airways inflammation (AAI) based on the adoptive transfer of Th2 polarised $CD4^+$ OVA-responsive T cells (OT-II cells). Challenging recipient mice with OVA via the airways leads to features of AAI. We have found that PIT, using a soluble peptide comprising the immunodominant OVA T cell epitope pOVA 323−339 given prior to allergen challenge, significantly reduces the disease severity. These findings were associated with fewer OT-II cells in the lungs, but unaltered numbers in lung draining lymph nodes (dLN), of mice that had received PIT compared to controls. In contrast to previous models which have utilised the transfer of naïve OT-II cells and where pOVA PIT has induced tolerance via a predominantly deletional mechanism, the frequency of OT-II cells was found to increase in blood, spleen and dLN following PIT in this model (prior to OVA challenge). Furthermore, OT-II cells from PIT treated, but not control mice, produced IL-10 in response to pOVA *in vitro*.

The effects seen after PIT in this model may be related to alterations in Th2 cell homing to the lung and are associated with substantial IL-10 production by OT-II cells following PIT. These findings may therefore hold relevance for the future translation of PIT to allergic patients.

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Regulatory T cell suppressive functions are not affected by cryo-preservation

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Regulatory T cells (Treg) offer an exciting potential therapy for the prevention and/or treatment of graft versus host disease and autoimmune disorders. A number of clinical trials using freshly isolated or expanded Treg have shown promising therapeutic benefit creating an opportunity for cryo-preserved Tregs to be used as an 'off the shelf' cellular reagent. In this study we investigated the effects of cryo-preservation on the suppressive properties of $CD4^+CD25^{High}Foxp3^+$ Treg.

Cytotoxic T ($CD8^+$) cells and Treg were isolated from LRS cones by RosetteSep and immuno-magnetic separation. Freshly expanded or cryo-preserved Treg were added into mixed lymphocyte reactions with autologous $CD8^+$ T cells and allogeneic monocyte derived dendritic cells. The suppressive properties of Treg were then measured by their ability to reduce the activation and proliferation of $CD8^+$ T cells. $CD8^+$ T cell surface activation markers were assessed by flow cytometry and proliferation by 3H-thymidine incorporation.

Preliminary results demonstrated that cryo-preservation of Treg had no effect on their ability to suppress the proliferation of alloreactive $CD8^+$ T cells. Proliferation was suppressed by $76 \pm 0.058\%$ and $79 \pm 0.061\%$ for Treg with or without cryo-perseveration. The early activation marker CD69 was suppressed by $71 \pm 0.034\%$ and $77 \pm 0.036\%$ and late activation marker CD25 expression was suppressed by $83 \pm 0.038\%$ and $84 \pm 0.041\%$ for freshly expanded and cryo-preserved Treg respectively.

These results demonstrated that there was no significant difference between the suppressive properties of freshly expanded Treg compared to cryo-preserved Treg, therefore confirming that regulatory T cell suppressive functions are not affected by cryo-preservation.

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A type 1 diabetes-associated CD25 haplotype affects $CD4^+CD25^+$ regulatory T cell function

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Numerous reports have demonstrated that $CD4^+CD25^+$ Tregs from individuals with a range of human autoimmune diseases, including Type 1 diabetes (T1D), are deficient in their ability to control autologous pro-inflammatory responses when compared to non-diseased, control individuals. Treg dysfunction could be a primary, causal event or may result from perturbations in the immune system during disease development. Polymorphisms in genes associated with Treg function, such as *IL2RA*, confer a higher risk of autoimmune disease. Although this suggests a primary role for defective Tregs in autoimmunity, a link between *IL2RA* gene polymorphisms and Treg function has not been examined. We addressed this by examining the impact of an *IL2RA* haplotype associated with T1D on Treg fitness and suppressive function. Studies were conducted using healthy human subjects to avoid any confounding effects of disease. We demonstrate that the presence of an autoimmune disease-associated *IL2RA* haplotype is associated with diminished IL-2-responsiveness in antigen-experienced CD4 T cells, as measured by phosphorylation of STAT5, which results in lower levels of FoxP3 expression by Tregs, and a reduction in their ability to suppress proliferation of autologous effector T cells. These data offer a rationale that accounts for the molecular and cellular mechanisms through which polymorphisms in the *IL2RA* gene impact upon immune regulation, and consequently upon susceptibility to autoimmune and inflammatory diseases.

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Manipulating Fc gamma receptor expression through macrophage polarization

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Monoclonal antibodies (mAb) have proven extremely successful in the treatment of a variety of malignancies. Despite this, responses remain variable and there is much scope for improvement. mAb activity is largely mediated by interactions between antibody Fc regions and Fcγ receptors on effector cells (predominantly macrophages). In addition, FcγR expression profile has been shown to modulate mAb efficacy: FcγRIIB is an inhibitory receptor, whilst human FcγRIIIA and its murine homolog FcγRIV are activatory, increasing effector cell function.

In light of these observations, much effort has been made to develop mAb with Fc regions engineered for higher affinity to activatory FcγR's. However these have only seen minor improvements in efficacy. We hypothesise that this may be due to inherent deficiencies in the resident effector cell population. Evidence suggests that tumour associated macrophages tend towards an anti-inflammatory, tumour promoting phenotype. It has been shown that it is possible, using selected Toll-Like Receptor agonists to polarize murine bone marrow derived macrophages towards an activatory FcγR phenotype. In order to assess whether these findings translate to humans, our study is now concerned with systematically characterising and comparing the FcγR expression profile of similarly stimulated human monocyte derived macrophages.

If reagents can be found that will safely skew tumour associated macrophages *in vivo*, then it is possible that these will increase effector cell recruitment when used in conjunction with currently available mAb, thus further improving patient treatment outcomes.

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Re-programming macrophages to enhance antibody immunotherapy

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Monoclonal antibodies (mAb) have become established in the treatment of a variety of malignancies – transforming patient outcomes. Despite this undoubted impact, responses remain variable and their mechanisms of action and of tumour resistance are controversial. Several strategies are being employed in an attempt to improve responses and one area in which there is growing interest is re-programming the tumour microenvironment to augment effector cell recruitment and function.

Antibody immunotherapy relies predominantly on activatory Fc-gamma-Receptors (FcγR) expressing macrophages for effector function. However, tumour associated macrophages have a pro-tumour, anti-inflammatory phenotype associated with a reduction in the activatory: inhibitory FcγR balance which we hypothesise reduces the potency of antibody therapy. The understanding of how macrophages are manipulated by tumours *in vivo* and how they may be re-polarised to augment mAb immunotherapy is a critical area of study where data is currently lacking.

Although previous studies have shown that macrophage polarity can be manipulated *in vitro* with characteristic phenotypic outcomes little has been done to correlate phenotypic changes with effector cell activity. Here we demonstrate using Toll-like Receptor agonists and other stimuli that we can efficiently polarise macrophage to an activatory FcγR phenotype both *in vitro* and *in vivo*. Further we show using our recently developed *in vitro* phagocytosis assay that these phenotypic changes lead to an enhancement of antibody mediated uptake of B cells. Finally, we demonstrate using an adoptive transfer model that we are able to use these clinically relevant reagents to enhance mAb mediated depletion of B cells *in vivo*.

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Electric fields – novel regulators of T helper cell activation and function

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Understanding the signals that control T cell responses is important for devising new therapies for autoimmune and inflammatory diseases. Small endogenous electrical fields (EFs) are generated naturally near tissue wounds and they accelerate healing of damaged tissue. Moreover, synthetic devices based on EFs are proposed to decrease infection and enhance healing in persistent wounds. The aim was to determine how physiological EFs (150 mV/mm) influence human T cell activation and polarisation. T cells, stimulated with anti-CD3/CD28, were exposed to EFs for 4 h and cultured for 1–3 days. EF exposure did not affect T cell viability or induce apoptosis. However, EFs caused a significant decrease in T cell proliferation and IL-2 secretion, indicating a dampening effect on early activation events. Importantly, EF exposure resulted in a significant decrease in secretion of Th1, Th17 and Treg cytokines IFNγ, IL-17 and IL-10 while IL-4 secretion, associated with Th2 polarisation, was reduced but not significantly. Although the level of the Th1, Th2 and Treg signature transcription factors T-bet, GATA-3 and FoxP3 were not markedly affected by EF exposure, the level of RORγt (Th17) was significantly reduced. Preliminary experiments indicate that the level of phosphoSTAT3, important forTh17 polarisation, was also reduced by exposure to EFs. These results provide important new insights into the effects of signals generated by physiological range EFs in dampening the activation and polarisation of T cells, especially Th17. They suggest EFs are a novel additional mechanism that can potentially be exploited to modulate immune-mediated injury.

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A rapid diagnostic test for human regulatory T cell function to enable regulatory T cell therapy

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Regulatory T cells ($CD4^+CD25^{hi}CD127^{lo}FOXP3^+$ T cells, 'Tregs') are a population of lymphocytes involved in the maintenance of self-tolerance. Abnormalities in function or number of Tregs are a feature of autoimmune diseases in man. The ability to expand functional Tregs *ex vivo* makes them ideal candidates for autologous cell therapy to treat human autoimmune diseases and to induce tolerance to transplants. Current tests of Treg function typically take up to 5 days, a kinetic disadvantage as clinical trials of Tregs will be critically dependent on the availability of rapid diagnostic tests before infusion into man. Here we evaluate a 7 h flow-cytometric assay for assessing Treg function, using suppression of the activation markers CD69 and CD154 on responder T cells ($CD4^+CD25^-$, 'Tresp'), compared to traditional assays involving inhibition of CFSE dilution and cytokine production. In both freshly isolated and *ex vivo* expanded Tregs, we describe excellent correlation with 'gold standard' suppressor cell assays. We propose that the kinetic advantage of the new assay may place it as the preferred rapid diagnostic test for the evaluation of Treg function in forthcoming clinical trials of cell therapy, enabling the translation of the large body of pre-clinical data into potentially useful treatments for human diseases.

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Mycobacteria activate γδ T-cell anti-tumour responses via cytokines from type 1 myeloid dendritic cells: a mechanism of action for cancer immunotherapy

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Attenuated and heat-killed mycobacteria display demonstrable activity against cancer in the clinic; however, the induced immune response is poorly characterised and potential biomarkers of response ill-defined. We investigated whether three mycobacterial preparations currently used in the clinic (BCG and heat-killed *M. vaccae* and *M. obuense*) can stimulate anti-tumour effector responses in human γδ T-cells. γδ T-cell responses were characterised by measuring cytokine production, expression of granzyme B and cytotoxicity against tumour target cells. Results show that γδ T-cells are activated by these mycobacterial preparations, as indicated by upregulation of activation marker expression and proliferation. Activated γδ T-cells display enhanced effector responses, as shown by upregulated granzyme B expression, production of the T_H1 cytokines IFN-γ and TNF-α, and enhanced degranulation in response to susceptible and zoledronate-treated resistant tumour cells. Moreover, γδ T-cell activation is induced by IL-12, IL-1β and TNF-α from circulating type 1 myeloid dendritic cells (DCs), but not from type 2 myeloid DCs or plasmacytoid DCs. Taken together, we show that BCG, *M. vaccae* and *M. obuense* induce γδ T-cell anti-tumour effector responses indirectly via a specific subset of circulating DCs and suggest a mechanism for the potential immunotherapeutic effects of BCG, *M. vaccae* and *M. obuense* in cancer.

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Immune tolerance post renal transplantation: role of soluble HLAG & T regulatory cells (CD4+CD25+Foxp3+)

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Background: Studying immune tolerance induced by regulatory T cells and HLA-G in kidney allograft acceptance may help in enhancing the understanding of its mechanisms.
Aim: To evaluate the role of soluble HLA-G (sHLA-G) and regulatory T cells (Treg) (CD4+CD25+ FOXP3+) in kidney graft success or failure in transplanted patients.
Subjects: Three groups were studied: kidney transplanted patients with no rejection episodes ($n = 56$); transplanted patients with biopsy-proven renal rejection ($n = 27$); healthy age-matched non transplanted individuals as controls ($n = 43$).
Methods: A quantitative sandwich ELISA assay and Flow cytometry techniques were used.
Results: The percentage of Treg was significantly lower in chronic rejection patients compared to control as well as graft stable groups. No significant difference was found between graft stable and control groups. In graft stable group, patients on Rapaimmune (RAPA) had a significantly higher Treg percentage compared to patients on Cyclosporine (CSA). The level of soluble HLAG was significantly higher in both transplanted patient groups compared to the control. Prograf, but not Cyclosporine or Rapaimmune, had positive effects on sHLA-G levels. Patients with chronic rejection had significantly lower mean level of sHLA-G compared to graft stable group. No relationship was found between donor type, infection or duration post transplant, and Treg percentage or sHLA-G levels.
Conclusion: The current study supports previous studies addressing the role of sHLA-G and Treg cells in inducing immune tolerance post kidney transplantation. We recommend to clinicians to include RAPA where possible in their immunosuppressive protocols.

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Probiotic strain *Lactobacillus casei* Shirota imprints a skin-homing profile on effector T-cells and exhibits dual mechanisms of immunoregulation

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Background: Interactions between host and intestinal microbiota play a crucial role in mucosal immune homeostasis. Probiotics confer health benefits via modulation of host immunity through effects on antigen-presenting cells. Dendritic cells (DC) dictate the type of T-cell immunity but also homing patterns of T-cells.
Methods: We characterized phenotype and function of human blood-enriched DC following conditioning with live, heat-killed, and secreted products of probiotic bacteria Lactobacillus casei Shirota (LcS).
Results: LcS in all cases conditioned DC to specifically induce skin-homing markers CLA and CCR4 on stimulated T-cells. While LcS-secreted products (LcS supernatants; LcS-SN) significantly reduced DC stimulatory capacity for allogeneic T-cells, there was no change in cytokine production by T-cells. However, live and heat-killed LcS significantly increased DC stimulatory capacity and specifically induced an immunoregulatory cytokine profile in stimulated T-cells. LcS-SN significantly increased DC expression of CD40 expression whilst live and heat-killed LcS significantly increased CD80 and CD83 expression. Toll-like receptors (TLRs) and HLA-DR were significantly reduced in both cases.
Discussion: Effects of probiotics on immune cell migration had previously not been investigated. Our novel data suggests the reported therapeutic benefits of LcS in inflammatory bowel diseases (IBD) may be due to induction of immunoregulatory properties in DC and T-cells, and diversion of effector T-cells away from intestinal sites, implications *in vivo* being reduction of intestinal inflammation. LcS biased DC towards immunoregulation via dual mechanisms of action; although LcS-SN did not alter T-cell cytokine production, the reduced stimulatory capacity of LcS-SN conditioned DC reflected that of tolerogenic human gut DC.

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Rapamycin favours the regulatory phenotype of antigen-specific Treg expanded from patients with autoimmune hepatitis by reducing the number of IFNγ+ cells

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Background: Autoimmune hepatitis type-2 (AIH-2), an inflammatory liver disorder, is associated with an impairment of regulatory T-cell (Treg) number and function. We have previously demonstrated that Tregs specific for CYP2D6, one of the main AIH-2 autoantigens, have greater suppressive ability on effector cell function than polyclonal Treg. The aim of the current study was to generate antigen-specific Treg and assess their phenotype over a 2 week period of expansion. The effect of rapamycin (RP), which can promote polyclonal Treg function, and of IL-6 and IL1-β, cytokines mimicking the pro-inflammatory milieu in patients, on antigen-specific Treg phenotype was also examined.

Methods: Antigen-specific Treg were generated by co-culture with CYP2D6-peptide-pulsed smDC. CYPD26-specific (CYP)-Treg were generated from 12 AIH-2 patients positive for the predisposing HLA-DR7/DR3 alleles; influenza-hemagglutinin-specific (HA)-Treg were generated from nine DR7+/DR3+ healthy subjects. CYP- and HA-Treg were expanded in the presence of: (i) IL-2 alone; (ii) IL-2 + RP; or (iii) IL-2 + IL6/IL1β. Flow-cytometric analysis was performed to evaluate Treg phenotype markers and intracellular cytokine production.

Results and conclusion: During expansion, CYP- and HA- Treg maintained the classical Treg phenotype (i.e. $CD25^{hi}$, $Foxp3^{+}$ and $CD127^{-}$), even when cultured in a pro-inflammatory environment. Compared to HA-Treg, CYP-Treg contained higher numbers of cytokine producing cells. Over the culture period, the frequency of IFNγ^{+} cells increased more in CYP- than in HA-Treg but was abrogated in both in the presence of RP. We have developed a protocol for the expansion of antigen-specific Treg, which could be utilised in the development of a Treg-based immunotherapy for AIH-2.

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Introduction of RAG1 and RAG2 influence the expression of recombination associated genes

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The present project explores a currently ignored possibility of using the naturally occurring recombinases, RAG1 and RAG2, to introduce a wanted transgene into the human immunoglobulin gene loci. RAG1 and RAG2 are the main players in generating the variable part of immunoglobulin molecules and T cell receptors. In order to perform their function properly they require certain recombination associated genes, like HMGB1 and HMGB2. In the hope of utilizing RAG enzymes as gene integrational tools, we have introduced these recombinases into a non-lymphoid RAG negative model cell line. Our study demonstrates that induction of RAG enzymes in a non-lymphoid cell line influences the expression of other genes associated with recombination. The two DNA binding HMGB1 and HMGB2, known to be vital for V(D)J recombination, decreases significantly upon RAG induction. This indicates that expression of RAG enzymes in excess in a non-lymphoid cell line may actually restrict the possibility of utilizing RAG enzymes as integrational tools.

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Weaning diet induces a sustained shift in metabolic phenotype and influences host immune response to supplementation with Bifidobacterium lactis NCC2818

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The process of weaning causes a major shift in intestinal microbiota and is a critical period in development of appropriate immune responses. Here we use a novel metabonomic approach, throughout the weaning period, to examine the responses of young piglets to novel dietary protein. In addition, we use this method to quantify differences between animal batches and assess physiological responses to probiotic supplementation. Six piglets were reared by their mothers for 24 h before removal to an SPF facility. At 21 days, all piglets were weaned onto an egg-based diet for a further 14 days. Similarly grouped siblings received *Bifidobacterium lactis* NCC2818 supplementation from 24 h onwards. This was repeated with a further six piglets, to allow the batch effects to be studied. The microbiota was analysed by 454 pyrosequencing, immunoglobulin production from immunologically relevant intestinal sites was quantified and the serum and urinary ^{1}H NMR metabolic profiles were obtained. A batch effect, presumably due to differences in up-take of environmental and microbial derived antigens during the first 24 h of life, was identified in all three analytic platforms. In addition, the modulatory effect of B. lactis supplementation could be identified in metabolic profiles and immune parameters, although it did not affect the composition of the microbiota. Here, for the first time, the urinary ^{1}H NMR metabolic profile was correlated to immunoglobulin production in this important test species. This multi-platform systems approach to early-life provides a method for uncovering non-invasive biomarkers of mucosal immune response, which has the potential for translation into human healthcare.

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FoxP3 expression in human stimulated T-cells is transient and dependent on T-cell density -still a valid marker for identification of human regulatory T-cells?

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Background: T-cell proliferation rates *in vitro* depend on factors including initial T-cell number, dose of stimulus, culture time and available physical space. FoxP3 is considered as the gold-standard marker to identify T-cells with a regulatory phenotype although its exact role in human T-cells remains controversial.
Methods: Proliferative T-cell responses and FoxP3 expression in human divided T-cells was assessed on CFSE-labelled T-cells cultured at different initial numbers of T-cells and antigen presenting cells (APC).
Results: T-cell proliferation rates depended on initial T-cell/APC numbers. Higher proliferation rates were achieved with lower T-cell/APC ratios, and decreased when initial T-cell numbers were increased. FoxP3 was expressed exclusively in virtually all divided T-cells when they had been cultured at high T-cell densities, irrespective of their CD4 expression and/or IL-10 content. At lower T-cell doses FoxP3 expression was not induced in divided T-cells even when most of the cells had undergone cell division. IL-10 content in T-cells was independent of FoxP3 expression. Induced FoxP3 expression in T-cells was subsequently lost in time when the stimulus was removed and T-cells were cultured at lower cell densities.
Discussion: FoxP3 expression in human T-cells is dependent on the environment, is transient and may not be an unequivocal marker for Treg. The results indicate that caution should be observed in human studies for using FoxP3+ as an indicator for Treg cells and highlight the possible disasters that could result if putative Tregs are used inappropriately *in vivo* in human immunotherapy.

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Intranasal tolerance induction is maintained in mice lacking CD11b but through different mechanisms

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Accumulating evidence suggests that complement is involved in the regulation of T cell responses. We have previously shown that in a minor H disparate skin transplantation model, mice deficient in complement C1q and C3 are resistant to tolerance induction after intranasal administration of HY ($H2A^b$/*Dby*) peptide. CD11b/CD18 (Complement Receptor 3 or Mac-1), is highly expressed on antigen-presenting cells, binds complement activation products (iC3b/C3b) and has been demonstrated to be required for orally induced peripheral tolerance to ovalbumin. We therefore hypothesised that CD11b was likely to mediate the complement effects and that CD11b-deficient mice ($CD11b^{-/-}$) would be resistant to tolerance induction after intranasal HY peptide administration. Surprisingly, indistinguishably from wild type (WT) females, indefinite survival of syngeneic male skin grafts was induced in $CD11b^{-/-}$ females following intranasal peptide treatment. However, the underlying mechanisms were different. Tolerant $CD11b^{-/-}$ mice displayed a defective antigen-specific $CD8^+$ T cell response compared with tolerant WT mice (HY-specific $CD8^+$ T cells in spleen: 4.3 ± 1.07% in WT versus 0.28 ± 0.1% in $CD11b^{-/-}$ mice, $P = 0.0037$). Moreover, after *in vitro* re-stimulation with male antigen, $Foxp3^+$ reglatory T cells from grafted $CD11b^{-/-}$ mice expanded significantly more than the regulatory T cells from the WT mice (WT: 1.52 ± 0.87% versus $CD11b^{-/-}$ mice: 4.35 ± 2.8%, $P = 0.0045$). CD11b-deficient dendritic cells also induced less proliferation and less Th1 differentiation of antigen-specific CD4 T cells. These preliminary data suggest that CD11b is not involved in the failure of tolerance induction in C1q- and C3-deficient mice and that different regulatory/tolerance mechanisms are induced in wild type and $CD11b^{-/-}$ mice.

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A large proportion of colorectal tumour-infiltrating CD4+ T cells are suppressive irrespective of Foxp3 expression

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The presence of increased numbers of $CD3^+$ T cells in colorectal cancer (CRC) correlates with improved prognosis. However, it is difficult to measure anti-tumour responses in tumour-infiltrating lymphocytes (TILs) suggesting these cells are suppressed. Although we have demonstrated $CD4^+Foxp3^+$ regulatory T cells (Tregs) within the tumour and its stroma, the numbers are often low. We sought to identify phenotypic and functional characteristics of $CD4^+Foxp3^-$ T cells to determine whether other regulatory populations exist within this environment.

Tumour samples were obtained from CRC patients with different stages of malignancy. Fixed tumour samples were examined by immunofluoresence for CD3, CD8 and FoxP3. TILs from fresh tumour tissue were stained with a panel of 20 antibodies (including Helios, LAG-3, LAP) and examined by FACS.

Histology revealed tumours to be infiltrated by $CD4^+$, $CD8^+$ and $Foxp3^+$ positive cells. Despite an increase in $CD4^+$ and $CD8^+$ T cells in advanced tumours, there was not always a concomitant increase in $Foxp3^+$ cells. Flow cytometry revealed the majority of the Treg fraction was $Helios^+$ (indicating thymically-derived) and expressed higher levels of CTLA-4 and CD39 than Tregs from colon and blood. However, 30% of 'conventional' $CD4^+Foxp3^-$ T cells express markers associated with Tregs including LAP (latency-associated peptide), LAG-3 and CD25 and were highly suppressive *in vitro*.

Tumour-infiltrating $CD4^+$ T cells are heterogeneous. A high percentage of these cells appear to have a regulatory function and include both $Foxp3^+$ as well as $FoxP3^-$ T cells. Overcoming the suppressive environment of CRC is a major challenge for boosting anti-tumour immunity.

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Functional consequences of IL1R2 expression by human T regulatory cells

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Background: T regulatory cells (Treg) have the ability to suppress autoimmune and allogeneic immune responses and are indispensable for the maintenance of self-tolerance. However, recent evidence suggests that proinflammatory signals may modulate Treg suppressive capacity. The proinflammatory cytokine, interleukin 1 beta (IL1 beta), which has been demonstrated to play an important role in Th17 versus Treg differentiation both in mouse and in human, signals through IL1R1, whereas IL1R2 serves as a decoy receptor. Here, we investigated the expression of IL1R2 on Treg and T effector cells (Teff) and tested the ability of soluble IL1R2 to modulate allogeneic immune responses.
Methods: Human $CD127^{lo}CD25^{+}CD4^{+}$ Treg and $CD127^{+}CD25^{-}CD4^{+}$ Teff cells have been sorted and *ex vivo* expanded with anti-CD3/anti-CD28 beads and recombinant IL-2. Expression of IL1R2 was investigated using flow cytometry and real-time PCR. Functional implications of expression of a decoy receptor have been tested using suppression assays and flow cytometry.
Results: IL1 decoy receptor is rapidly upregulated by Treg after TCR stimulation, with more than 85% of Treg expressing IL1R2, compared to <15% of Teff cells upregulating its expression. In addition, soluble IL1R2 is released into Treg culture medium. Importantly, blocking of IL1R2 partially impairs Treg suppressive activity and recombinant IL1R2 is able to inhibit an allogeneic immune response.

Our results demonstrate a novel decoy strategy utilized by Treg to ensure regulation in the proinflammatory environment.

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The potential of Tregs with indirect allo-specificity to induce transplantation tolerance

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Regulatory T cells (Tregs) are a functionally and phenotypically distinct lymphocyte subset (1−5% of $CD4^{+}$ T cells). Adoptive transfer of $CD4^{+}CD25^{+}FoxP3^{+}$ Tregs has been shown to induce tolerance in autoimmunity and prevent transplant rejection. In murine models we demonstrated that alloantigen-specific Tregs are functionally superior compared to polyclonal Tregs in inducing transplant tolerance and the indirect allospecificity is necessary to prevent chronic vasculopathy. To establish human Tregs with indirect allospecificities is challenging. The main reason is that the majority of Tregs cultured in the presence of autologous dendritic cells pulsed with alloantigens are specific for self-antigens. To generate and expand large numbers of human alloantigen-specific Tregs suitable for clinical use, erythroleukemic K562 cells engineered to express CD64 and CD86 (obtained from Carl June) were co-transfected with expression vectors encoding HLA-DRα and HLA-DR1β*0101 molecule with covalently linked the HLA-A2 peptide. The same K64.86 cell line has been also engineered to co-express HLA-DRα and HLA-DR1β*0101 chains. The K64.86-DR1/A2 or K64.86-DR1 cell lines will be used as artificial antigen presenting cells (aAPCs) to generate Tregs with indirect allospecificity for HLA-A2 peptide and restricted by HLA-DR1. To study their purity, safety and function *in vivo*, alloantigen-specific Tregs will be then co-injected with human PBMCs in a SCID/NOD$\gamma c^{-/-}$ mouse model of human skin transplantion and compared to Tregs with direct allospecificity. The data obtained form this study will pave the way for the selection of the best alloantigen specific Tregs for cell therapy in solid organ transplantation.

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Inhibition of PI3Kd significantly inhibits inflammation and immunoglobulin production in the chronic house dust mite model of allergic airway inflammation

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Asthma is a chronic inflammatory disease characterized by infiltration of activated immune and inflammatory cells including eosinophils and neutrophils, to the airways, which is believed to be orchestrated by Th2 cells. Chronic exposure of mice to inhaled house dust mite extract (HDM) results in inflammation of the airways that mimics certain key features of asthma. Here we investigated the effect of PI3Kδ inhibition on airway inflammation and immunoglobulin responses in the chronic HDM model.

Male Balb/c (n = 8/group) mice were orally dosed with 1, 3, 10 mg/kg PI3Kδ inhibitor or vehicle from day -1. Mice were challenged intranasally with PBS or HDM four times per week for 5 weeks. At the end of week 5 mice were sacrificed and BALF and lung tissue were collected for analysis of eosinophils, neutrophils and T cells by flow cytometry. Serum was taken by cardiac puncture to analyse the levels of house dust mite specific IgG isotypes and IgE.

A significant, dose dependent, decrease in eosinophils (90%), neutrophils (72%), $CD4^{+}$ T cells (69%) and $CD8^{+}$ T(84%) cells in the BALF and a significant decrease in eosinophils (75%) and neutrophils (85%) in the lung digest was obtained. A significant reduction in house dust mite specific immunoglobulins (Ig) IgG1 (87%), IgG2a (44%), IgG2b (63%) and IgE (85%) was also observed.

The data indicates that the PI3Kδinhibition results in a reduction of airway and lung tissue eosinophils, neutrophils, $CD4^{+}$ T cells, $CD8^{+}$ T cells and reduces the production of HDM specific immunoglobulins. These results suggest that PI3Kδ inhibition could be of therapeutic benefit in the treatment of asthma.

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Post-traumatic immunosuppression syndrome and its reversal by salvaged blood transfusion

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Immunity against infection is profoundly suppressed after major trauma and surgery but is poorly defined and mechanisms involved in its' generation incompletely understood. Using total knee arthroplasty (TKA) as a model, we previously characterized this syndrome in terms of decreased NK levels and IFN-γ synthesis; and also showed allogeneic blood transfusion was detrimental (*Lancet* 2004;363:1025–30). We now report changes in macrophage activation and cytokine profiles with and without re-infusion of postoperatively salvaged blood.

Among 43 TKA recipients, 25 received salvaged blood transfusion and 18 were untransfused postoperatively. No patients received allogeneic blood. Salvaged blood was collected into Acid-Citrate-Dextrose (ACD) and re-infused within 6 h using Dideco-797 recovery device. Peripheral blood was collected preoperatively and 2–5 days postoperatively. Plasma pairs were assayed by ELISA for Neopterin and by Flow-cytometric Bead Array for 12 cytokines.

Results showed that, whereas non-transfused patients showed no change in postoperative Neopterin levels, they were significantly elevated in patients receiving salvaged blood ($P = 0.002$). In non-transfused patients cytokine profiles showed postoperative decreases in pro-inflammatory (IFN-γ, TNF-α, IL-2, IL-17, IL-1β) and increases in anti-inflammatory cytokines (IL-4, IL-5, IL-13, IL-10) – a pattern that was completely reversed in salvaged blood transfused patients. IL-6 and IL-22 increased and IL-9 decreased postoperatively in both groups.

Thus, post-traumatic immunosuppressive syndrome persisted at least to 5 days after TKA, rendering patients vulnerable to hospital-acquired infections. This syndrome was completely reversed by ACD salvaged blood re-infused within 6 h post-operatively. Underlying molecular mechanisms and broader clinical exploitation of this intriguing immunostimulatory effect merit further investigation.

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Scavenger receptor regulation of Macrophages in the tumor microenvironment

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Scavenger receptors (SR) are expressed on distinct subsets of macrophages (MΦ), in the spleen marginal zone, the lymph nodes, lung and peritoneum. SRs are also expressed on Tumor-Associated Macrophages (TAM) which are recruited by tumors to support tumor growth and progression. The microenvironment induces a phenotypic shift from a pro-inflammatory M1, to an anti-inflammatory pro-tumor M2 phenotype. This project investigates the involvement of SRs in regulating the function of TAMs in tumorigenesis. SRs will also be used as targets for tumor therapy to modulate TAMs into an anti-tumor response. Both induced (B16 melanoma) and spontaneous (MMTV-PyMT mammary carcinoma) murine tumor models will be used. As a starting point we stained stroma from these tumors with SR antibodies and found evidence for similarity of phenotype to macrophages in the spleen. Consequently, we have injected wt mice with anti-SR antibodies and studied the response. We find elevated levels of TNF as well as lower ability of spleen cells to secret IL-10. This suggests that engagement of specific SRs on MΦs can be used to regulate local inflammation. To support this we find that adjacent B cells in the spleen respond by down regulation of complement receptors. We are currently continuing these studies in the tumor models and will evaluate if targeted immunomodulation of TAMs could provide a novel method for cancer therapy.

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Suppressors of cytokine signalling (SOCS) 2 and 3 diametrically control macrophage polarisation

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M1 macrophages, induced by pro-inflammatory stimuli, and involved in the acute response. M2 macrophages are polarised by anti-inflammatory stimuli and mainly involved in healing. The Suppressors of cytokine signalling (SOCS) are important regulators of both LPS and cytokine responses but their role in macrophage polarisation is unknown. Myeloid restricted SOCS3 deletion (SOCS3LysMcre) resulted in profound resistance to endotoxic shock, whereas SOCS2–/– mice were highly susceptible. This was associated with striking bias towards M2-like macrophages in SOCS3LysMcre mice, whereas the M1-like population was enriched in SOCS2–/– mice. Through adoptive transfer experiments we show that these antipodal responses to endotoxic shock and to polymicrobial sepsis (caecal ligation puncture) were both transferable and entirely macrophage-dependent. Critically this dichotomous response was associated with enhanced T-reg recruitment by SOCS3–/– cells, yet in the presence of SOCS2–/– macrophages, Foxp3+ T cells were completely absent at the inflammatory site. The altered polarisation coincided with enhanced IFNγ- induced STAT1 in SOCS2–/– macrophages and enhanced IL-4/IL-13 induced STAT6 phosphorylation in SOCS3–/– cells corresponding to altered binding to traditional gene markers of M1 and M2 macrophages (iNOS, TNFα, ARG-1 and CCL-17,). In the absence of SOCS2, macrophages seem unable to elicit an anti- inflammatory response even when stimulated with typical M2 stimulus (IL-4/IL-13, IL-10), whilst the absence of SOCS3 prevents a pro-inflammatory response even in the presence of LPS/IFNγ. Interestingly, the polarisation of macrophages in the absence of SOCS2 or SOCS3 seems fixed and irreversible. Therefore SOCS are essential controllers of macrophage polarisation and regulate the inflammatory response.

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Human protective and regulatory T cell responses during experimental respiratory syncytial virus infection

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Respiratory syncytial virus (RSV) is the major cause of viral bronchiolitis, the leading cause of hospitalisation in infants and is increasingly recognised as a cause of respiratory disease in the elderly. Bronchiolitis is in large part caused by an excessive inflammatory response to infection with inflammation causing poor gas exchange. Murine studies show that regulatory T cells (Tregs) regulate the immune response to primary RSV infection by controlling the influx of inflammatory cells in the lungs and airway, limiting the production of inflammatory cytokines and chemokines. Whilst mouse models have revealed much about the mechanisms of pathology they do not reproduce all aspects of human disease. Studies of natural infection in infancy are problematic due to the difficulty of obtaining appropriate samples.

We therefore performed experimental infection of 12 unselected healthy adult volunteers with an identical intranasal dose of RSV A Memphis 37. Subjects' ages were 19–34 years; seven were male. Symptom scores and samples from the upper respiratory tract and peripheral blood were obtained daily, to profile the clinical, virological, and immunological kinetics of infection. Four subjects suffered symptomatic upper respiratory tract infection, with RSV confirmed by culture in three. PCR revealed a further three asymptomatic infections. Immune parameters (including prior antiviral immunity and measurement of Treg responses by flow-cytometry of peripheral blood lymphocytes and ELISpot) will be correlated with the diverse outcomes observed. Findings from this study provide further insights into how RSV-induced inflammation is controlled in adults, with possible implications for vaccine development.

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Roles for IL-10 in RSV infection

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Interleukin (IL-) 10 is a pleiotropic cytokine with broad immunosuppressive functions, particularly at mucosal sites (e.g. intestine and lung). Some pathogens may boost host IL-10 production in order to evade immune defenses.

Respiratory syncytial virus (RSV) is a leading global cause of severe lower respiratory tract infections in infants and is associated with recurrent wheeze in later life. Despite limited viral diversity, immunity to re-infection is partial and often ineffective, suggesting that RSV circumvents the host's immune protective mechanisms.

We found that infection of BALB/c mice with human A2 strain of RSV induces IL-10 production by CD4+ and CD8+ T cells in the airways and that a proportion of these cells co-produce IFN-γ. Furthermore, IL-10-deficient mice infected with RSV succumb to more severe disease, with enhanced weight loss, delayed recovery and greater cell infiltration of the respiratory tract, accompanied by pronounced airway neutrophilia and heightened levels of pro-inflammatory cytokines and chemokines in the bronchoalveolar lavage fluid. Additionally, the proportion of IFN-γ-producing T cells was enhanced in the lungs suggesting that IL-10 acts to dampen effector T cell responses, although viral load was similar in IL-10-deficient mice and controls. Similar findings were noted in mice treated with anti-IL-10R antibody and infected with RSV.

Therefore, host IL-10 production inhibits disease and inflammation in mice infected with RSV but does appear to not support viral persistence. These findings highlight new insights into the mechanisms of immune regulation in the respiratory tract during viral disease.

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Antiviral therapy reduces virus-specific immunity and improves immune function in elderly mice with chronic herpesvirus infection

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Herpesviruses such as cytomegalovirus (CMV) establish chronic infection and induce strong adaptive immune responses. CMV-specific T cell responses have been demonstrated to accumulate with age, a phenomenon termed memory inflation. The accumulation of CMV-specific CD8 T cells is correlated with worse vaccination outcomes and an increased risk of mortality in elderly donors. Intervention to suppress CMV-specific T cell responses is of therapeutic value although the importance of antigen load in memory inflation is uncertain. Chronic murine CMV (MCMV) infection also causes inflation of MCMV-specific T-cells that display a highly differentiated phenotype indicative of repeated antigen exposure. In order to assess whether antiviral treatment could modulate the MCMV immune response we set up a study in a mouse system. Chronic MCMV infection was established in C57BL/6 mice and high-doses of valacyclovir were administered to the animals for 12 months to suppress viral replication. MCMV-specific immunity was then assessed; our data shows that antiviral treatment significantly reduced the frequency of MCMV-specific CD8 T-cells by 20%, as assessed by intracellular cytokine staining for five immunodominant MCMV-epitopes, and corresponded to a 30% increase in naïve CD8 T-cells in the spleen. Treatment of MCMV also reduced the morbidity and mortality associated with influenza challenge in MCMV infected mice. This was seen by elevated numbers of influenza-specific CD8 T cells in the caudal mediastinal lymph node during acute influenza infection. In summary these data suggest that antiviral therapy may be highly effective in improving immune function in older CMV seropositive donors.

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CD200 receptor signaling promotes the establishment of virus persistence in mucosal tissue through inhibition of innate immunity

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Mucosal surfaces are critical ports of entry and/or exit for numerous pathogenic viruses that establish chronic infection, including herpesviruses. The salivary glands represent the major site of persistent replication and shedding of murine cytomegalovirus (MCMV). CD200 receptor (CD200R) is a negative regulator of peripheral and mucosal immunity. Viruses including some herpesviruses target the CD200R signalling pathway through acquisition of functional CD200 homologues. However, the role that CD200R signalling plays in regulation of immunity during persistent viral infection *in vivo* is unclear. We now report that the CD200-CD200R pathway suppresses innate and adaptive antiviral immunity during MCMV infection. CD200R-deficient mice exhibited elevated NK cell accumulation during acute MCMV infection that corresponded with improved control of virus replication in the spleen. CD200R−/− mice also exhibited a dramatic increase in CD4 T cell and monocyte/macrophage accumulation, and enhanced NK cell cytotoxicity in the salivary glands during the persistent phase of infection that, critically, resulted in decreased virus replication. Depletion of CD4 T cells, which afford protection from MCMV persistence, did not abrogate control of virus replication by CD200R−/− mice. Critically, therapeutic blockade of CD200 with monoclonal antibody during persistent infection of RAG-deficient mice also improved control of virus replication, demonstrating that CD200R inhibits innate immune responses that afford protection from cytomegalovirus persistence irrespective of adaptive immunity. These results uncover CD200R as a critical regulator of antiviral immunity during cytomegalovirus persistence and highlight the potential therapeutic importance of promoting innate, as well as adaptive, immune responses during chronic virus infections.

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The immune visibility of tumour cells can be altered upon *in vitro* culture with chemotherapy drugs

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In addition to the tumour cell ablating properties of chemotherapies, some have also shown remarkable capacity to modulate the immune system. Gemcitabine (GEM), is one such chemotherapy. It has been shown to affect numbers of regulatory T-cells and myeloid derived suppressor cells in human and in mouse and has shown promising synergy with dendritic cell vaccination. Initial studies from our group suggest that GEM can influence expression of cell surface molecules important for the efficient surveillance and effector function of the immune system. We have shown that GEM can increase the expression of human leukocyte antigen (HLA) class I, Leukocyte immunoglobulin-like receptor subfamily B1 and CD95 on a number of tumour cell lines from different origins in short term *in vitro* culture. This effect occurs on cells surviving GEM treatment. Culturing tumour cells with GEM increased beta-2-microglobulin expression but did not alter cellular HLA heavy chain concentration as assessed by Western blot. In addition to the increase of surface HLA class I and presumed subsequent quantitative increase in antigen presentation, preliminary data suggest that qualitative changes may also occur. Specifically, components of the antigen presentation machinery are altered, indicating that neo- or cryptic-epitopes may be generated and the immune response to tumour strengthened. This is yet to be investigated fully but current data may explain effects seen in previous *in vivo* studies and lends further credence to the idea that chemotherapy and immunotherapy should be used in combination.

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Identification of a putative soluble isoform of CD200 in human mesenchymal stem cells

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Human mesenchymal stem cells (hMSCs) are multipotent cells capable of differentiating into several cell types including chondrocytes, adipocytes and osteoblasts *in vitro* and *in vivo*. In addition to their function as supporting cells in the bone marrow, hMSCs are known for their prominent immunomodulatory abilities for which the underlying mechanisms remains to be clarified. CD200 is a transmembrane glycoprotein expressed by a variety of cell types and has been shown to be an important immunoregulatory molecule through its interaction with the inhibitory CD200 receptor expressed by cells of the myeloid lineage and T cells. We demonstrate here that hMSCs express all three transcripts of CD200. Stimulation of hMSCs with the pro-inflammatory cytokines IFN-γ and TNF-α increases the expression of all CD200 transcripts. Cell surface expression of CD200 was studied by flow cytometry and staining of cells was performed with two commercially available CD200 antibodies derived from two different clones. No cell surface expression of CD200 was detected on hMSCs with either antibody. However, after permeabilization we observed a signal intracellularly with one of the two CD200 antibodies tested. The intracellular protein was also present in NK cells, but absent in T cells and monocytes. The absence of cell surface CD200 expression on hMSCs demonstrated here differ from results obtained in other studies. We do, however, detect a signal intracellularly indicating the presence of a putative soluble isoform of CD200 which could be involved in an immunomodulatory mechanism.

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Treatment of tumor cells with low concentration of 5-Fu hinders the sensitization of dendritic cells

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Chemotherapy is the standard treatment for pacients with most types of cancer and 5-fluorouracil (5-Fu) is one of the first line drugs for colorectal cancer. However, chemotherapy based on maximum tolerable dosage is frequently associated with severe side effects. We previously observed that *in vitro* exposition of tumor cells to antineoplasic agents, in low, non citotoxic concentrations make them more immunogenic, while exposing dendritic cells (DC) to these antineoplasic agents can increase their ability to induce *in vitro* antitumor response. In the present study we aimed to verify the *in vivo* effect of the pre-treatment of tumor cells with a non cytotoxic concentration of 5-Fu. For this purpose, we s.c. inoculated C57/Bl-6 mice with MC-38 colorectal tumor cells and 7 days later they were vaccinated with wild type or 5-FU-treated, tumor lysate-pulsed DC (DC/5-Fu). Evaluation of tumor growth showed that the phenomena observed *in vitro* are not associated with protective effects *in vivo*, since the animals treated with DC/5-Fu vaccine showed increased growth of tumor (670.91 mm^2 at the 30th day) when compared with the control group (234.38 mm^2). We will further evaluate whether such treatment is inducing the development of Treg or MDSC. Financial support: Fapesp 2009/18331-8.

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Immunomodulatory molecules from *Fasciola hepatica* directly suppress activation of IL-17-producing $\gamma\delta$ and CD4 T cells that mediate autoimmune disease

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Epidemiological studies have demonstrated a reduced incidence of allergy and autoimmune diseases in individuals infected with helminth parasites. We have previously reported that infection of mice with the helminth parasite *Fasciola hepatica* exerts bystander suppression of autoantigen-specific Th17 cells that mediate autoimmunity. In the present study we have examined the effect of excretory secretory (ES) products from *F. hepatica* on activation and function of IL-17-secreting T cells. We demonstrated that systemic administration of ES from *F. hepatica* significantly attenuated the clinical symptoms of experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis. This was associated with reduced IL-17 and IFN-γ production and proliferation of Th1 and Th17 cells. We also observed that ES-treated mice had a lower frequency of $\gamma\delta$ T cells, which are known to play a pivotal pathogenic role early in EAE. Furthermore, administration of ES significantly suppressed innate IL-17 production by $\gamma\delta$ T cells induced *in vivo* following injection of IL-1β and IL-23. An investigation of the mechanism involved revealed that ES suppressed the APC function of dendritic cells (DC), but also render $CD4^+$ T cells unresponsive to signals that trigger proliferation and subsequent cytokine production, such as anti-CD3, PMA or ConA. The direct effect on T cells was not associated with cell death, but could be reversed by inhibition of reactive oxygen species (ROS) with *N*-acetyl cysteine. Our findings demonstrate that products of a helmith parasite can both indirectly and directly suppress the function of IL-17-secreting T cells that mediate autoimmune disease.

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Pregnancy in Lupus patients: different but not impossible

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Systemic Lupus Erythematous is a chronic multi-system autoimmune disease that affects mostly women of childbearing age. The risk of complications and adverse fetal outcomes is higher than in the general population, and the management of flare-ups can be complex during this period. Advancing technology and better understanding of pregnancy and lupus interactions have improved maternal and fetal outcomes over the last four decades.

The outcome for mother and child is best in women with inactive and stable systemic lupus erythematosus for at least 6 months before pregnancy and when kidney disease is in remission. The main risk factors for adverse pregnancy course and outcome are disease activity, hypertension, nephritis with proteinuria and maternal serum antibodies to SS-A/Ro, SS-B/La, cardiolipin, beta2-glycoprotein I and lupus anticoagulant. The patients need to cooperate with obstetricians and physicians and undergo intense surveillance throughout the gestation period for optimal disease control. We retrospectively evaluated the prevalence, clinical manifestation, treatment strategies, and maternal and fetal complications of the pregnant women with systemic lupus erythematosus who were followed in our department.

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Serum 25(OH)D levels can predict Foxp3+ treg frequency and steroid responsiveness in severe asthmatics

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Vitamin D insufficiency is highly prevalent, with a recent study showing that during the winter and spring months 87% of the UK population is vitamin D insufficient. Vitamin D insufficiency has been linked to several autoimmune diseases. More recently vitamin D sufficiency has been implicated in the maintenance of pulmonary health. As part of an ongoing double-blinded, placebo-controlled clinical trial, our lab is investigating the capacity of calcitriol, the active form of vitamin D, to restore clinical steroid responsiveness in Steroid Refractory asthma. This provides the unique opportunity to analyse well-defined Steroid Sensitive (SS) and Steroid Refractory severe asthma patients from the initial screening visits. Preliminary results have shown that SR patients have a lower frequency of CD4+Foxp3+ T cells in the peripheral blood compared to SS. Following a 2-week course of Prednisolone a significant reduction in the frequency of Regulatory T cells in the peripheral blood of SR asthmatics, but not SS asthmatics was observed. Concordant with this SS asthmatics had a higher basal level of serum 25-hydroxy-vitamin D (25(OH)D) as compared to SR. A strong correlation between serum 25(OH)D and the frequency of Foxp3+ T regulatory cells in the peripheral blood of severe asthmatics was observed. Parallel laboratory investigations are investigating the mechanisms by which vitamin D modulates Foxp3+ Treg frequencies. These data support a role for vitamin D in maintaining Foxp3+Treg frequencies, and suggest serum 25(OH)D levels may predict the frequency of FoxP3+ T cells and steroid responsiveness in severe asthma.

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Vitamin D intervention for MS: a pilot study to assess immunomodulatory activity

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An extensive body of *in vitro* evidence has shown that aside from its role in calcium metabolism, vitamin D exerts significant immunomodulatory effects. Epidemiological studies indicate that MS patients have lower levels of serum vitamin D compared with healthy controls, and vitamin D levels have been inversely associated with disease activity. Furthermore, vitamin D can attenuate experimental autoimmune encephalomyelitis. This has raised the question of whether vitamin D supplementation may be beneficial in MS. However, the effects of vitamin D supplementation have not been tested in properly controlled clinical trials, and it is unclear what effects it would have on the immune system. As a prelude to a clinical trial we performed a pilot study in healthy individuals to examine the immunomodulatory effects of vitamin D supplementation. After 15 weeks of supplementation with 5000 IU/day of vitamin D3, serum 25(OH) vitamin D levels rose significantly from baseline, corresponding with a striking increase in IL-10 production by peripheral blood mononuclear cells in response to a variety of stimuli. Furthermore, IL-17 production by CD4 T cells was significantly reduced after supplementation with vitamin D. Previous studies have reported anti-inflammatory effects of vitamin D using relatively high doses of active 1,25(OH) vitamin D *in vitro* or *in vivo* in mouse models. Our findings are consistent with these studies, but show for the first time that vitamin D intervention in human subjects exerts measureable anti-inflammatory effects *in vivo*, and provide a strong case for assessing the effects of vitamin D supplementation in clinical trials.

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Defining immune mechanisms and dose-relationships that limit the safety of immunomodulatory biotherapeutics

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A rapid increase in the development of immunomodulatory biologics by the pharmaceutical industry has occurred in recent years. Immunomodulatory biologics targeting the T cell molecules Programmed Death-1 (PD-1) and Lymphocyte-activation gene 3 (LAG-3) are examples of such drug development activity. The clinical indications for targeting PD-1 and for LAG-3 are predominantly viral infections and cancers. Current preclinical safety testing of such immunomodulatory biologics relies on standard toxicity studies which do not completely provide the dose relationships for immunological safety, in particular the dose windows for emergence of autoimmunity. There is a need for experimental system/s that can identify dose-response windows for both efficacy and potential autoreactivity. We have used a transgenic TCR model to examine dose-relationships of biologics that target PD-1 and LAG-3 in the context of T cell responses to specific antigen (immunity) or to a surrogate self-antigen (autoreactivity). The TCR transgenic system is the F5 TCR bearing T cell which recognises a peptide, NP68 derived from the influenza virus nucleoprotein. We have used a related peptide, NP34 which is a partial agonist to the F5 TCR and represents a surrogate self-peptide. Using this system in the presence of LAG-3 and/or PD-1 blocking antibodies we demonstrate dose-response windows for potential reactivity to self.

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Expansion of highly suppressive and non-plastic human regulatory T cells in the presence of rapamycin and all-trans-retinoic acid

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Treating autoimmune diseases and promoting transplant tolerance using *ex vivo* expanded human $CD4^+CD25^+FOXP3^+$ T regulatory cells (Tregs) is now feasible. To characterize the optimal conditions for expanding clinical-grade Tregs *in vitro*, with respect to Treg phenotype, function and plasticity, we investigated the effect of rapamycin (RAPA) and/or all-trans-retinoic acid (ATRA) addition to Treg cultures. RAPA and ATRA enhanced suppressive function of Tregs that was maintained even after drug-withdrawal from cultures. The two treatments induced unique patterns of surface molecules expression, with RAPA enhancing expression of skin-homing and ATRA inducing gut-homing chemokine receptors and PD-L1. However ATRA permitted expression of both IL-17 and IFN-γ, in contrast to RAPA, which did not. The combination of RAPA+ATRA expanded Tregs with both skin and gut-homing characteristics, highest suppressive ability and minimal plasticity. Finally starting to define which therapies should be used together with Tregs in transplant recipients, we investigated the effect of immunosuppressive drugs as tacrolimus, mycophenolate (MPA) and methylprednisolone on Treg phenotype and suppressive ability. The results showed that RAPA− and/or ATRA-treated Tregs were affected differently by individual drugs and the exposure to MPA caused the most significant amount of cell death.

We conclude that RAPA and RAPA+ATRA are effective treatments for expanding clinical-grade human Tregs, inducing homing receptors conditioning Tregs to access sites of inflammation and T cell priming and inhibiting plasticity. Likewise we show that the immunosuppressive regimen currently used in preventing allograft rejection can affect Treg viability and the success of Treg immunotherapy.

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Novel strategies to enhance current antibiotic therapies

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Current treatment options for personnel exposed to bacterial BW agents predominantly involve the use of licensed antibiotics. These treatments work very effectively when used shortly after exposure and, ideally, before the symptoms of disease associated with infection begin to emerge. The timing of the decision to take these treatments is therefore important to the overall effectiveness of these medical countermeasures (MedCMs). This decision is primarily informed by a 'triggering event' such as a detector signalling the presence of a BW agent. However, if this information is not available the first 'triggering event' may be the symptoms associated with infection. Initiating treatment at this point (post-symptomatically) is likely to be less effective, putting personnel at risk of developing a life-threatening disease. We are evaluating a novel therapeutic strategy which aims to make existing MedCMs more effective and which may make it possible to extend the timeframe within which antibiotics can be administered. This project aims to establish whether the use of specific anti-inflammatory compounds, that target fundamental immune pathways, can be used to enhance the overall effectiveness of antibiotics by controlling the unbridled immune response typically found during the course of infection with the bacterium *Francisella tularensis*.

Anti-HMGB1 antibody, which targets the nuclear protein released by cells following injury or infection, has been assessed during *in vivo* models of infection.

Preliminary data suggests that this compound modulates the inflammatory response and may be beneficial in enhancing the effectiveness of antibiotics in treating the disease tularaemia.

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Association of serum anti-Tn IgM with breast cancer recurrence

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Approximately 13 000 women succumb to breast cancer (BCa) in the UK each year, mainly as a result of development of secondary cancers, metastases. There is a need for the identification of biomarkers and new approaches for treating the disease. Elevated levels of tumour associated carbohydrate antigens (TACA) including the Tn-antigen (GalNAcα1-Ser/Thr) have been shown to be associated with poor prognosis BCa.

The levels of serum IgM interacting with a neoglycoprotein carrying the Tn-antigen was assessed using serum from a multicentre study on factors influencing BCa metastasis (the DietCompLyf study). Anti-Tn IgM from patients with recurrent ($n = 12$) and non-recurrent ($n = 12$) BCa was assessed. The patient samples were collected approximately 1 year post diagnosis and patients were followed up for 5 years. Decreased levels of anti-Tn IgM (as a ratio of total IgM) was observed in sera from BCa patients with recurrent disease (Mann–Whitney U, $P = 0.04$). The specificity of the IgM binding to Tn-antigen was characterised by inhibition using relevant and irrelevant monosaccharides. The immunoglobulins in sera from recurrent and non-recurrent BCa cases were profiled on a glycoarray platform (Consortium for Functional Glycomics, CFG v4.1) consisting of 465 unique glycan structures. The analysis identified significant differences in serum IgG binding to glycans in the conformation GlcNAcα1-3Galβ1-4GlcNAcβ, GlcNAcβ1-6GalNAcα, and Galα1-2Galβ were noted.

Further investigations extending this work are on-going in our laboratories. Taken together these findings suggest that the humoral response to TACA may be of value for monitoring BCa patients at high risk of disease recurrence post-surgical intervention.

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A specialized tube to make RosetteSep™ enrichment of specific cell subsets faster and easier

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Many experimental protocols require the enrichment of specific cell subsets from peripheral blood. RosetteSep™ cell enrichment and standard mononuclear cell (MNC) preparation both involve density gradient centrifugation, which entails slowly layering the sample over the density gradient medium to avoid mixing, and carefully pipetting to remove the enriched cells after centrifugation. Centrifugation must be performed with the brake off to avoid disturbing the enriched cell layer, further lengthening the process.

SepMate™, a centrifugation tube with a specialized insert, was developed to allow rapid layering of the sample onto the density gradient medium, and pouring off of the enriched cells after centrifugation, thus simplifying the entire process. Furthermore, when using SepMate™, the cocktail incubation time and centrifugation time could each be shortened to 10 min, making RosetteSep™ cell enrichment even faster. RosetteSep™ enrichments of mononuclear cell subsets using the SepMate™ tubes and protocol gave equivalent purity and recovery of desired cells compared to using the standard RosetteSep™ protocol, and desired cells could be enriched from whole blood in <30 min. Purities obtained using RosetteSep™ with the SepMate™ tube were: CD3 T Cells 96 ± 1 ($n = 5$), CD4 T Cells 94 ± 5 ($n = 3$), CD8 T Cells 85 ± 11 ($n = 4$), B Cells 92 ± 6 ($n = 3$), NK Cells 85 ± 5 ($n = 5$), and monocytes 68 ± 8 ($n = 4$). The protocol is easily scalable to process multiple samples simultaneously, and the SepMate™ tube can also be used to prepare MNCs.

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The success of tumour immunotherapy following depletion of Foxp3+ regulatory T cells depends on tumour size and infiltration of sufficient numbers of activated T cells

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Selective depletion of Foxp3$^+$ regulatory T (Treg) cells can result in immune-mediated control of tumours. Here we show that Treg cell depletion in mice with palpable carcinogen-induced tumors results in reduced tumour growth and tumour regression in some mice. We have been using this model to compare the immunological differences which exist within progressing versus regressing tumours. Whilst T cell activation was observed in all treated mice, success of the treatment depended on tumor size at the start of treatment and the number of IFNγ-producing T cells. Furthermore, our study reveals a concordance between tumour control and the number of T cells in the tumour. These data pinpoint lymphocyte recruitment and tumour infiltration as critical factors in defining the success of Treg cell depletion immunotherapy. We are currently characterizing the mechanisms underpinning successful infiltration of T cells into tumors with the aim of improving existing T cell based immunotherapies.

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Effects of vitamin D deficiency during early life on the development of neonatal house dust mite induced allergic airways disease

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Vitamin D levels during pregnancy have been associated with reduced wheeze and asthma in infants. Additionally, vitamin D has a profound effect on immune function, promoting T regulatory cell (TReg) populations with suppressive properties including IL-10 secretion. The mechanisms underlying vitamin D insufficiency during pregnancy and asthma susceptibility in offspring remain unknown; we aimed to define the immune responses underlying this relationship. Pregnant mice were fed either a vitamin D deficient or normal chow diet from day 16 gestation. Intranasal HDM or saline was administered intermittently to their pups from day 3 of life for 6 weeks. Pups were weaned on to either a vitamin D deficient or normal chow at 3 weeks of age. Airway hyperresponsiveness to methacholine was measured using the forced oscillation technique, and flow cytometry used to assess cell populations in the lung at 6 weeks of age. Maternal vitamin D insufficiency during pregnancy caused significant increases in Th2 (CD4+T1ST2+) cells (61.61 versus 20.78×10^3 cells/ml; median, $P = 0.019$) and decreases in CD4+IL-10+ TRegs (1.09 versus 3.70×10^3 cells/ml; median, $P = 0.0043$) in the lungs of vitamin D deficient HDM-exposed pups, compared to pups from vitamin D sufficient mothers. This was true for all pups born to vitamin D deficient mothers regardless of allergic status. No differences were detected in any parameters of lung function or total inflammation. This study suggests maternal vitamin D status is important in immune regulation in the neonate, yet vitamin D deficiency alone is not sufficient to increase the severity of neonatal allergic airways disease.

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Biological therapy in rheumatoid arthritis: experience of an internal medicine department

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Rheumatoid arthritis is a systemic autoimmune disease of unknown etiology characterized by symmetric and erosive synovitis. The course of Rheumatoid arthritis is usually chronic and progressive leading to irreversible joint deformities and functional impairment.

Non-steroidal anti-inflammatory drugs, disease modifying anti-rheumatic drugs and low-dose corticosteroids have been used for the treatments of Rheumatoid arthritis.

Recently it has been stressed the importance of an early start of disease modifying anti-rheumatic drugs, however a significant amount of patients do not respond or do not tolerate these drugs. The advances in molecular biology have given rise to a new treatment modality, the biological therapy. These agents are drugs, usually proteins, which can influence chronic immune dysregulation resulting in chronic arthritis. According to the mechanism of action these drugs include: anti-TNF drugs, IL-1 blocking drugs, IL-6 blocking drugs, agents blocking selective co-stimulation, CD 20 blocking drugs. Biologic agents have revolutionized the treatment of Rheumatoid arthritis, producing significant improvement in clinical, radiographic, and functional outcomes not seen before. Nowadays the treatment for rheumatoid arthritis is based on early diagnosis, early aggressive therapy with optimal doses of disease modifying anti-rheumatic drugs and, if no improvement has been achieved in a period of 6 months, early introduction of biologic drugs. In this paper we report our department's experience, evolution of biological therapy and its responses in patients with Rheumatoid arthritis since 2005. This evaluation will be based on patients' symptoms, DAS-28, radiological changes, analytical markers (rheumatoid factors, anti-citrulinated protein antibodies, erythrocyte sedimentation rate, C-reactive protein) and complications.

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Safe antigens: their use in immune sera production and vaccine approach

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Fatal accidental pathologies as scorpion envenoming are known to be a public health problem in tropical and subtropical regions of the world. In these regions at-risk, immunotherapy remains the main used approach to treat envenomed patients. Optimization of this therapy could be approached by safe use of attenuated antigens to produce efficient antibodies and also in vaccine development.

Detoxification of toxic antigens using dose rate of 765 Gy/h of 2 kGy-gamma radiation successfully abolished toxicity without reducing their immunogenic properties. Immunoprotective properties of detoxified antigens were evaluated against the lethal effects of venom at medium and long-terms. Vaccinated mice were protected from the toxic effects of native venom doses at 1, 3 and 6 months after immunization schedule. Mice were protected against a challenge of 4 LD_{50} doses of native venom, 1 month after immunization. This protective effect was improved and effective at 3 and 6 months and immunized animals were protected respectively against 6 and 10 LD_{50} of native venom

Isotype evaluation of IgG1 and IgG2 titer immunsera was assessed to Identify response pathways (Th1−Th2) to better understand the immunological response. It appears that native venom induced higher IgG1 titer, indicating the predominance of a Th2 type response. However, the irradiated one produces higher titer of IgG2, suggesting that Th1 cells are predominantly involved in the immune response, more specifically in macrophage activation. These data suggest that after protein irradiation, an antigen, known to induce Th2 response, is able to switch the immune system towards a Th1 pattern.

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A critical role for antigen dose in peptide immunotherapy of autoimmune disease

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The gold standard for the treatment of autoimmune disease is the induction of antigen-specific tolerance towards the eliciting antigen, without the perturbation of normal immune function. Previous work from this laboratory has shown that repetitive intranasal administration of a myelin basic protein peptide, MBP Ac1-9[4Y], induces protective tolerance in the TCR transgenic Tg4 EAE model, characterised by the induction of IL-10 secretion and an anergic, regulatory phenotype in Th-1 cells. The influence of route and dose of antigen on the outcome of peptide immunotherapy was explored. Tolerance induction by the subcutaneous (s.c.) route was closely related to peptide dose, with higher s.c. peptide doses inducing an anergic, suppressive CD4+ T cell phenotype, suppression of IL-2 and IFN-gamma and enhanced IL-10. However, higher s.c. peptide doses also carried the risk of adverse effects during the initial phase of treatment, where high levels of inflammatory cytokines were detected. Novel application of dose escalation for self-peptide immunotherapy allowed s.c. administration of high MBP Ac1-9[4Y] doses without adverse effects, retaining the potent tolerogenic capacity of high peptide doses and providing protection from EAE. Peptide dose escalation in Tg4 Rag-1 deficient mice specifically modulated the response of a monoclonal CD4+ T cell population upon subsequent exposure to strong TCR stimulation. Gene expression profiling is underway to dissect the mechanism of dose escalation, which will have implications for the translation of self-peptide immunotherapy into the clinic.

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Progesterone modulates maternal CD8 T cell function – a potential role in recurrent miscarriage therapy

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Background: Recurrent miscarriage (RM), the consecutive loss of three or more pregnancies, occurs in 1% of couples. In the majority of cases there is no identifiable cause but a maternal immunological response to the allogenic fetus has been implicated. Progesterone (P4) is currently being trialled as a therapeutic agent in women with RM and is suggested to have immunomodulatory properties.
Objective: We have investigated the maternal CD8 T cell immune response to fetal antigens in women with RM and the immunomodulatory effect of progesterone on cytokine production by CD8 T-cells.
Results: HLA-peptide dextramers detected a fetal (HY) antigen specific CD8 T cell response in 40% (8/20) of women with RM. This is the first identification of fetal (HY) specific T cells in women with RM.

The effect of P4 at a range of concentrations on maternal T cells following stimulation by PHA was studied. IFNγ production was significantly reduced by P4 at 10 μM. Using fetal antigen specific T-cell clones it was also demonstrated that P4 significantly reduced IFNγ release in response to fetal antigen (Median 36% reduction, $P \leq 0.001$).
Conclusion: Fetal specific CD8 T cells can be detected in women with a history of recurrent miscarriage. P4 can modulate the production of IFNγ in maternal T cells, including fetal specific T cells. Further experiments are investigating the cellular mechanism of action of P4. Greater understanding may aid selection of RM patients who would benefit from P4 therapy and the rational design of better therapeutic strategies.

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The effect of carvacrol on the induction of tolerogenic dendritic cells

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Previously it has been shown that carvacrol, the major compound of many *Oreganum* species, can prevent inflammatory damage in a mouse model of rheumatoid arthritis. The exact mechanism of immune regulation by carvacrol remains to be clarified, but it is suggested that dendritic cells (DCs) might play a role. DCs are able to induce antigen specific regulatory T cells by presenting peptides to T cells in a tolerogenic state. Here we investigated whether carvacrol can induce DCs with a tolerogenic phenotype and function.

A micro array was performed to study the effect of carvacrol in combination with heat stress (HS) and HS alone on the DC. A set of about 50 differentially expressed immunological relevant genes was obtained, but no clear tolerogenic expression profile was found. On protein level carvacrol-HS and HS treated DCs are less mature compared to untreated DCs, which indicates a more tolerogenic DC. More importantly, these DCs induce a less activated and less pro-inflammatory antigen specific T cell that has increased expression levels of the regulatory T cell marker Foxp3. Finally, carvacrol-HS treated DCs can give a functional suppression of proteoglycan induced arthritis. In conclusion, HS and carvacrol-HS treatment can indeed induce a tolerogenic phenotype in DCs and *in vivo*, carvacrol HS treated these DCs are able to reduce PGIA.

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PLGA, PLGA-TMC and TMC-TPP nanoparticles differentially modulate the outcome of nasal vaccination by inducing tolerance or enhancing humoral immunity

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Development of vaccines in autoimmune diseases has received wide attention over the last decade. However, many vaccines showed limited clinical efficacy. To enhance vaccine efficacy in infectious diseases, biocompatible and biodegradable polymeric nanoparticles have gained interest as antigen delivery systems.

We investigated in mice whether antigen-encapsulated PLGA (poly-lactic-co-glycolic acid), PLGA-TMC (*N*-trimethyl chitosan) or TMC-TPP (tri-polyphosphate) nanoparticles can also be used to modulate the immunological outcome after nasal vaccination.

These three nanoparticles enhanced the antigen presentation by dendritic cells, as shown by increased *in vitro* and *in vivo* $CD4^+$ T-cell proliferation. However, only nasal PLGA nanoparticles were found to induce an immunoregulatory response as shown by enhanced Foxp3 expression in the nasopharynx associated lymphoid tissue and cervical lymph nodes. Nasal administration of OVA-containing PLGA particle resulted in functional suppression of an OVA-specific Th-1 mediated delayed-type hypersensitivity reaction, while TMC-TPP nanoparticles induced humoral immunity, which coincided with the enhanced generation of OVA-specific B-cells in the cervical lymph nodes. Intranasal treatment with Hsp70-mB29a peptide-loaded PLGA nanoparticles suppressed proteoglycan-induced arthritis, leading to a significant reduction of disease.

We have uncovered a role for PLGA nanoparticles to enhance $CD4^+$ T-cell mediated immunomodulation after nasal application. The exploitation of this differential regulation of nanoparticles to modulate nasal immune responses can lead to innovative vaccine development for prophylactic or therapeutic vaccination in infectious or autoimmune diseases.

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The activation state of apoptotic cells differentially modulates antigen-induced arthritis

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Previously we have demonstrated that apoptotic cells suppressed disease in antigen-induced arthritis (AIA), a model of inflammatory arthritis. Here, we investigated whether the activation state of the apoptotic cell influenced their capacity to modulate inflammation.

Activation of dendritic cells prior to induction of apoptosis (aAC) abolished their ability to suppress AIA when compared to resting AC ($P < 0.0001$). Forty-eight hours after intravenous adminstration, AC, but not aAC, significantly increased TGF-beta production by dendritic cells (DC) ($P < 0.01$) and B cells ($P < 0.01$) and led to the expansion of the CD4+Foxp3+ Treg population in the spleen ($P < 0.0001$). *In vitro*, both AC and aAC induced IL-10 production by peritoneal macrophages, but the latter led to a greater production of IL-12 and TNF ($P < 0.05$). Transfer of DC from mice that had received AC, but not aAC, suppressed the development of arthritis in recipient mice.

In conclusion, the suppressive properties of apoptotic dendritic cells are dependent upon their prior activation state. Furthermore resident DC mediate this suppressive effect possibly via the induction of TGF-beta and CD4+ regulatory T cells.

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IL-10-secreting regulatory T cells 'self-regulate' differentiated, effector T helper (Th) 1 populations

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Interleukin (IL)-10 is a potent anti-inflammatory cytokine produced by multiple cell lineages. It is now well-recognised in both murine and human cells that IL-10 can be co-secreted with IFN-g by Th1 cells in both infectious and tolerant settings. We have previously shown that repeated intranasal administration of a high affinity analogue of the acetylated N-terminus myelin basic protein (MBP) peptide 1–9 (Ac1–9) to mice transgenic for the TCR recognising MBP Ac1–9 (Tg4 mouse; B10.PL, $H\text{-}2^u$) induces IL-10-secreting regulatory T cells (IL-10 Tregs). These IL-10 Tregs express the master Th1 lineage transcription factor T-bet, indicating their Th1 origin. While both Th1 and Th17 cells have been implicated as autoimmune-inducing cell populations, we have previously demonstrated in our Tg4 model that Th1 cells transfer experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, more effectively than Th17 cells. Here, we demonstrate that IL-10 Tregs suppress both the proliferation and cytokine secretion of differentiated Th1 and Th17 cells. In addition, Th1 cells differentiated in the presence of IL-10 Tregs transfer less severe experimental autoimmune encephalomyelitis (EAE) to Tg4 recipients relative to Th1 control cells transferred alone. We further demonstrate that IL-10 Tregs suppress the cycling of differentiating effector Th1 and Th17 cells, and also induce the Th1 cells that do cycle to co-secrete IL-10 with IFN-g. Taken together, these data imply a mechanism of infectious tolerance whereby Th1-derived IL-10 Tregs 'self-regulate' differentiating Th1 cells both by impairing their proliferative capacity and inducing their secretion of the IL-10 regulatory cytokine.

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Assessing the immunogenic and protective potential of different viral vector regimes, in the mouse model of tuberculosis infection

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Control of tuberculosis (TB) remains one of the most serious challenges to global health. With bacillus Calmette-Guérin (BCG) being an unsatisfactory vaccine and multi- and extensive- drug resistant (MDR/XDR) TB being on the rise, there is an urgent need for the development of a more efficacious vaccine. Current vaccination strategies aim to either replace or boost BCG. Viral vectors induce strong cellular responses and have been shown to successfully boost prior BCG immunity and further improve its protective efficacy in different pre-clinical animal models. The most representative being MVA85A, modified vaccinia Ankara virus expressing antigen 85A, currently the most advanced TB candidate vaccine in clinical trials. Another promising viral vector system is based on recombinant adenoviruses.

In this study, a new replication-deficient recombinant simian adenoviral vector, sAd85A, was evaluated for its immunogenicity and protective potential, alone or in combination with MVA85A. Intradermal administration of sAd85A, induced strong antigen specific CD4+ and CD8+ T cell responses, when administered in an A-M (sAd85A followed by MVA85A) or in a B-A-M vaccination strategy (BCG followed by A-M.). In mice, when sAd85A and MVA85A were administered systemically in a BCG – sAd85A – MVA85A regimen, no improvement over BCG alone was detected on M.tb challenge. However, a single intranasal immunisation with sAd85A provided significant protection against aerosol Mycobacterium tuberculosis challenge compared to naive animals.

Further work will evaluate the BCG boosting potential of i.n. sAd85A when administered alone or when boosted further by MVA85A.

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A major regulatory role of NKG2D ligands in transplantation

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The self-encoded 'stress-ligands' MICA (human) and Rae-1 (mouse) play fundamental roles in immune responses to viruses, tumors, and possibly allergens. However, there remains some uncertainty over their roles in allograft rejection. Using a skin graft model, we unexpectedly found that chronic graft expression of Rae-1 can prolong graft survival across a major donor/recipient allo-mismatch. We propose that this is because chronic Rae-1 expression exerts local immune suppression that substantively delays systemic infiltration. Consistent with this, the direct immune response driven by cells of the graft is suppressed, while the indirect response, driven by recipient APCs presenting minor antigens, is largely normal. Our data emphasize that, even at the level of single molecule up-regulation, cell stress is sensed by the immune system with profound consequences for survival or rejection of grafted tissue.

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Immunomodulation by dental pulp mesenchymal stem cells (SHED) is mediated by Indoleamine 2,3-dioxygenase

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Mesenchymal stem cells are the focus of much interest due to their potential applications in regenerative medicine, and more recently for their ability to regulate immune responses. Much of the work to date has been performed using bone marrow derived mesenchymal stem cells (bmMSC). Stem cells from human exfoliated deciduous teeth (SHED) have been identified as an easily accessible alternative source of mesenchymal stem cells. Here we demonstrate that SHED are comparable to bmMSC in their immunomodulatory properties. SHED where found to be analogous to bmMSC in their lack of expression of activatory co-stimulatory molecules CD40, CD80, and CD86. Similarly, both cells populations where found to express the negative co-stimulators PD-L1 and PD-L2. Co-culture of SHED or bmMSC with aCD3/CD28-activated PBMC demonstrated that both cell populations are equivalent in their ability to inhibit polyclonally activated allogeneic T-cell proliferation and to induce $FoxP3^+$ regulatory T-cells. In both cases inhibition of the tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) reversed these effects. In the case of inhibition of proliferation IDO was demonstrated to function through the depletion of tryptophan. Addition of exogenous tryptophan reverses the inhibitory effects of IFN-g activated SHED conditioned medium upon T-cell proliferation. These findings highlight IDO as the key mediator of immunomodulation by MSC and demonstrate that SHED provide an easily accessible alternative source of cells for use in immunomodulatory therapies.

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CD137 for Isolation and expansion of Ag-specific T cells using Dynabeads(R)

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A protocol has been developed for the isolation of expandable, viable and functionally intact antigen-specific CD8+ T cells. Dynabeads® FlowComp™ technology isolates activated antigen-specific T cells by use of an agonistic anti-CD137 antibody conjugated to a modified biotin and nitrated streptavidin coated Dynabeads®. The modified biotin and nitrated streptavidin facilitates a gentle release mechanism and the procedure enables isolation of bead-free antigen- specific T cells. For further expansion of the isolated cells, Dynabeads® Human T-Activator which are magnetic beads conjugated with agonistic antibodies specific for CD3, CD28 and CD137 were used.

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Ex vivo expansion of tumor- reactive T cells using Dynabeads® Mouse T-Activator CD3/CD28/CD137 enhances therapeutic efficacy

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Adoptive transfer of antigen-specific T lymphocytes is a promising therapy for cancer and chronic infections. Application of Dynabeads® CD3/CD28 in clinical trials has resulted in polyclonal expansion of large numbers of effector T cells capable of tumor destruction in adoptive immunotherapy. We reported previously that co-stimulation of T cells *in vitro* through CD137 by a soluble anti-CD137 mAb in addition to CD3/CD28 activation significantly enhanced T cell proliferation and survival. Furthermore, concomitant administration of agonist anti-CD137 mAb significantly enhanced the therapeutic efficacy of transferred T cells or DC vaccines. These data suggest that further improvements of the expansion protocol involving CD137 signaling pathway may enable the generation of more potent effector T cells.

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B cell activating factor (BAFF) binding receptors (BBR) on B cells in patients with rheumatoid arthritis (RA) after rituximab

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Aim: To investigate BBR expression in relation to clinical relapse following B cell depletion with Rituximab (RTX).
Methods: BAFF-R, TACI and BCMA expression on PBMC were performed using combinations of CD19, CD38 and IgD (%) in five healthy controls (HC), 11 patients pre-RTX, and 11 patients relapsing Concordant with B cell repopulation (CR) and 11 relapsing >3 months after repopulation (Discordant: DR). Significance levels was 5%.
Results: *Phenotype*: CR patients had lower % of naïve mature B cells compared to DR patients (54% versus 79%) but higher % of plasmablasts (15.4% versus 2.3%). % post-GC B-cells was uniformly decreased after RTX (CR: 1.9%, DR: 1.5%), compared to pre-RTX (21.2%).
BBR expression: Percentage of transitional and naïve B cells expressing BAFF-R was lower in all RA patients after RTX (CR and DR), compared to HC. CR patients showed lower % of transitional (27.4%) than RA pre-RTX (73%), DR (53.9%) and HC (93.9%). TACI on post GC B cells was lower for CR (50.6%) and DR (54.4%) versus HC (89.8%). BCMA expression was similar in all groups. BAFF levels rose following RTX in all patients.
Conclusion: Patients CR relapsed with higher percentage of plasmablasts and lower BAFFR on naïve B cells. Patients DR relapsed with higher naïve B cells, although BAFF-R expression was lower. TACI was uniformly low after RTX. Increased plasmablasts in CR patients suggests that differentiation into antibody-producing cells may relate to relapse. Low TACI low expression and BAFF-R/BCMA dysregulation may underly delayed acquisition of memory B cells in DR patients.

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Epigenetic regulation of immunoglobulin class switching

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The enzyme Activation Induced cytidine Deaminase (AID) plays a critical role in the maturation of the vertebrate immune response, initiating somatic hypermutation and immunoglobulin class switch recombination. AID functions by deaminating cytidine bases in the Ig genes. This act introduces base-pairing mis-matches into the DNA and initiates various pathways that lead to error prone repair (as in the case of somatic hypermutation) or the formation of DNA breaks that can undergo recombination (class switching). However, if inappropriately targeted AID can lead to cancer causing mutations and translocations. To date, little is known about the mechanisms that direct AID action to the immunoglobulin genes and protect the rest of the genome from its action. We have used chromatin immuno-precipitation and genome wide sequencing (ChIP-seq) technology to determine the genomic distribution of AID in human B cells activated to undergo class switching. This analysis reveals that AID is widely distributed across the genome. In light of this finding we have investigated the epigenetic landscape at these sites to gain insight into the specific factors and histone modifications that might be involved in directing AID action and the DNA repair activities that regulate its impact on the genome.

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Genome-wide analysis of modified histones in human Th1 and Th2 cells

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Analysis of histone and DNA modifications in chromatin from differentiated T cell subtypes has shown that many loci are differentially organized in different T cell subtypes. Examples of this are genes encoding specific signature cytokines or transcription factors that drive their expression. Recent data has begun to highlight the histone modifications that demarcate regulatory domains such as enhancers and locus control regions. In this study, we have studied chromatin organization in highly polarized *ex vivo* differentiated human Th1 and Th2 cells. Unsurprisingly, the organization of putative enhancers relative to a particular transcription start site is complex. In particular, regions of silenced chromatin, marked by H3K27me3 enrichment, are not as extensive over differentially expressed human genes as has been demonstrated in murine T cell differentiation models, suggesting fundamental differences in this process between mouse and man. There are significant differences between Th1 and Th2 cells in marking of specific enhancers. We have begun to map the sites of recruitment of transcription factors such as the orphan nuclear hormone receptor NR4A3 onto this landscape, this data demonstrates recruitment of NR4A3 to putative enhancers.

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Evaluating the effect of BCG vaccination on a whole-blood mycobacterial growth inhibition assay (BACTEC MGIT)

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Most childhood vaccines generate an antibody response that correlates strongly with protection and is easy to quantify. However, protection from tuberculosis (TB) is likely to be complex involving the coordinated activity of multiple cell types, and the mechanisms involved have not been fully elucidated. TB vaccine studies to date have used IFN-γ as the main immunological readout, but this may not be reliable. There is much interest in identifying a valid, strong correlate of protection which can help distinguish between candidate vaccines.

In this study, the BACTEC MGIT assay was evaluated for its reproducibility, transferability and ability to detect a BCG vaccine induced response in both BCG-naïve and -vaccinated individuals. Intra-subject variability is low between cultures for the same visit, but is greater between repeated pre-vaccination visits. This may be partially due to biological variability, as IFN-γ responses to PPD also show variability. In the previously BCG vaccinated group, there is no significant difference in growth inhibition following a second vaccination. However, in the BCG naïve group, there is a significant reduction in mycobacterial growth at 8 weeks post-vaccination. Net growth correlates with ELISpot IFN-γ response pre-vaccination, but this relationship is lost post-vaccination.

Future work will assess two further mycobacterial growth inhibition assays on the same samples with the aim of evaluating and comparing these assays. The findings from this study could contribute to the harmonisation and standardisation of different mycobacterial assays of growth inhibition, allowing comparative evaluation of the immunogenicity and efficacy of future novel TB vaccine candidates.

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Antigen-specific regulatory T cells against heat shock protein-70 suppress experimental rheumatoid arthritis

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Directing regulatory T cells (Tregs) for suppression of autoimmune diseases like rheumatoid arthritis (RA) is currently under heavy investigation. However, clinical application has been hampered by the unknown nature of the disease inducing antigens in autoimmunity. Heat shock proteins (Hsp) are ubiquitous self-antigens that are overexpressed in inflamed tissue. Interestingly, Hsp70 (-derived peptides) can prevent the induction of RA in several models for autoimmunity. Therefore, we hypothesized that T cell epitopes of Hsp70 can be targets for epitope-specific immunotherapy in inflammatory diseases via the activation of antigen-specific Tregs. By expanding already existing Hsp70-specific Tregs via immunizing donor mice, we were able to generate sufficient amounts of primary antigen-specific Tregs for adoptive transfer therapy. One therapeutic administration of Hsp70-specific Tregs in recipients 3 weeks after disease induction significantly suppressed experimental arthritis, probably due to *in vivo* activation by Hsp70. This hypothesis was confirmed by the lack of suppression of transferred Ova-specific Tregs, for which the cognate antigen is not expressed in the recipients. Transferred Tregs were found in draining lymph nodes and joints up to 50 days after transfer and remained FoxP3+. Phenotypical analysis of Tregs from Hsp70-immunized mice, or *in vitro* restimulated Tregs showed enhanced expression of LAG-3 and neuropillin-1, which suggests that the mechanism of suppression comes from the interaction with antigen-presenting cells. Thus, we show that antigen-specific Tregs are potent suppressors of established inflammation. Therefore, Tregs against self antigens associated with inflammation could be suitable candidates for adoptive transfer therapy in autoimmune diseases with unidentified disease causing antigens.

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Protective subunit of *Bacillus anthracis* enhances human dendritic cell activation, reduces dendritic cell production of anti-inflammatory cytokines and enhances T-cell stimulation

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Background: Anthrax is caused by spores of *Bacillus anthracis*, and has sparked interest over the past two decades due to it's potential use as a biological weapon. The recombinant protective antigen (rPA) mediates entry of the toxin into host target cells, and represents the basis for current anthrax vaccines. We aimed to investigate the effects of rPA on human dendritic cells (DC), unique in their ability to induce primary immune responses.
Methods: The effects of conditioning human blood DC with rPA, and heat-killed Bacillus cereus (B.cer) as a positive control, were analysed by flow cytometry. DC were conditioned with culture medium only as a negative control.
Results: rPA significantly upregulated expression of DC activation markers CD40, CD80 and lymph-node-homing marker CCR7. DC production of anti-inflammatory cytokines IL-10 and TGFβ were significantly downregulated upon rPA conditioning. Production of inflammatory cytokines IL-12 and IL-6 was unchanged. There were no significant differences upon B.cer conditioning in any experiments. We optimised a system to analyse proliferation of DC-stimulated T-cells in a primary *in vitro* response; rPA increased the stimulatory capacity of DC compared with B.cer or basal medium conditioned DC.
Conclusions: *Ex vivo* treatment of DC with rPA may provide the basis for antigen-specific protection against anthrax and may cut down the time to reach cell-mediated immunity.
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Avonex immunomodulatory effects in MS patients

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Interferons are used in Multiple sclerosis patients with the aim to reduce MS specific symptoms and to modify the disease process. Interferons may influence to the patients immune answer. We analysed 49 MS patients immune answer during the immunomodulation with Avonex (Biogen). Thirty-six patients had cerebrospinal and 13 cerebral MS remiting relapsing form. Average age in the group was 34 years. We analysed Avonex influence on whole blood cells level, lymphocyte subpopulations CD4+, CD8+, CD16+ cell and immunoglobulin G,A,M level. Lymphocyte subpopulations were analysed using BD Facs Calibur laser flow cytofluorimeter. Humoral immunity was checked by Nephelometer BN II. Bab antibodies were determined by using ELISA test system produced by Buhlmann Laboratories (Swiththerland). Leucopenia was determined in 6%, neutropenia in 4%, lymphopenia in 25%.of patients. Bab antibodies were determined in 9.5% of patients. Avonex treatment reduce mainly CD4+ cell level in 20%, CD 8+ in 16% and CD 16+ cell level in 14% of MS patients. Immunoglobulin level was with very waild variety among the patients in the group. Avonex main influence was observed by reduced lymphocyte count, especially CD4+ cells and CD 8+, CD 16+ cells. Bab antibody positivity was not stable during follow up time.Avonex mainly act on cellular immune parameters decreasing TH1 immune answer.

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Carbon nanoparticles activate the NLRP3 inflammasome and efficiently target draining lymph nodes following injection

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Recent studies have highlighted the potential of carbon nanoparticles as drug carriers and cancer targeting systems. Carbon Nano-Onions (CNOs) are amongst the first carbon polymorphs to have been described; yet, they have not been widely investigated, particularly in terms of their immunomodulatory properties. Here, we demonstrate that small CNOs (5 nm ± 1 diameter) are efficiently taken up by antigen-presenting cells and selectively promote the secretion of interleukin 1(IL-1) alpha and beta. The enhancing effect of CNOs on secretion of IL-1β by mouse dendritic cells was dependent on the NLRP3 inflammasome. Importantly, activation of the inflammasome by CNOs can be attenuated by covalent addition of benzoyl-carboxylic functional groups. In a peritonitis model, CNOs induced the production of the inflammatory cytokine IL-6 and promoted the recruitment of neutrophils, eosinophils and mast cells. Remarkably, and in striking contrast with carbon nanotubes – a class of carbon allotropes highly documented for their physicochemical properties, our data indicate that CNOs can migrate rapidly from sites of injection to mouse draining lymph nodes.

This study is the first to characterize the immunomodulatory properties of small CNOs, and supports their application as novel immunomodulatory and targeting agents.

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Rituximab for child with chronic relapsing autoimmune hemolytic anemia

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Aim: Warm-type idiopathic autoimmune hemolytic anemia (AIHA) is a relatively common hematologic disorder resulting from autoantibody production against red blood cells. Steroids represent the first-line therapeutic option, and immunosuppressive agents as well as splenectomy are used for refractory cases. Recently, the anti-CD20 monoclonal antibody rituximab has been shown to control autoimmune hemolysis in patients with refractory chronic disease.
Method: We report results from a retrospective analysis of five child patients receiving rituximab for steroid-refractory AIHA of the warm type at a mean age of 9 year (range 3–14 year). All patients were given methyl-prednisolone as first-line treatment and some of them also received azathioprine and intravenous immunoglobulin. All patients were considered refractory to steroids and/or immunosuppressive drugs and all were given weekly rituximab (375 mg/m^2) for 4 weeks.
Results: Two patients required packed red cell transfusions before starting rituximab and all became transfusion-free. At a mean follow-up of 432.4 days (range 240–892 days) since the treatment of AIHA with rituximab, all patients are alive, and all of them in complete remission (CR) and two patients had combs' test positive. In
Conclusion: Our study shows that anti-CD20 rituximab is an effective and safe alternative treatment option for idiopathic AIHA, in particular, for steroid-refractory disease.

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Oxygen therapy with more precaution in immune deficient patients so double percaution is needed in neonates and o2sat not be morethan 96%

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Oxygen attacks harmful substances in the body in normal dosage and in normal patients and O_2 therapy is most effective way of sustain health but in immune deficient patients it doesnot work right and so in these patients we should get more percausion

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A common programme of proliferative control for diverse homeostatic responses by T cells

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Regulation by homeostatic mechanisms ensures that the number and functional diversity of peripheral T cells is maintained at a constant level. In lymphopenia induced proliferation (LIP), naive T cells are induced to undergo cell division to restore the homeostatic balance. The clonal response to lymphopenia is extremely diverse. Here, we have compared LIP of two distinct T cell clones: OT-I cells undergo rapid division accompanied by differentiation, whereas F5 cells divide slowly and remain naive. We adopted a mathematical modelling approach to determine whether this heterogeneity represents distinct mechanisms of cell cycle control, or if a common mechanism can account for such diversity. *In silico* testing of different models of T cell proliferation revealed that LIP of both T cell clones was best described by a two-compartment model of stochastic single divisions, albeit with distinct model parameters. Constraining the two compartment model with cell number and cell cycle data resulted in a model that was sensitive to cell density, and predicted key biological parameters of LIP, including the homeostatic set point. Thus, the diverse and heterogeneous nature of the clonal T cell response to lymphopenia could be accounted for by a single common model of cell cycle control.

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IL-2 engineered nanoAPC effectively activate viral specific T cells from chronic HBV infected patients

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Impaired function of virus specific T cells resulting from virus persistence is one of major mechanisms underlying the development of chronic hepatitis B viral infection. Previously, we found that IL-2 can restore the effector function of T cells rendered tolerant by antigen persistence. However, systemic administration of IL-2 induces organ pathology and expansion of Treg cells. Here, we show that nano-antigen presenting cells (nanoAPC) with engineered HLA alleles and IL-2 deliver peptide-MHC complexes (pMHC), costimulatory molecules and IL-2 to antigen specific T cells resulting in enhanced expression of CD25 expression and activation of TCR signalling pathways, with while suppression of PD-1 expression on viral responding CD8 T cells from chronic HBV patients. The enhanced activation of CD4 and CD8 T cells induced by IL-2-nanoAPC was antigen dependent and IL-2-nanoAPC did not affect Treg cells. At a size of 500 nm, the nanoAPC effectively induce immune synapse formation on antigen specific T cells and accumulate as free particles in the lymphoid organs. These attributes of IL-2-nanoAPC or other bio-adjuvant engineered nanoAPC have profound implications for their use as a therapeutic strategy in the treatment of chronic HBV infection or other chronic viral diseases.

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Extraimmunization among Iraqi children

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Children who received more than the recommended number of doses of any vaccine before 2 years of age were considered to have received *Extra-immunization dose*. The aims of this study are to determine the frequency and percent of this type of doses among child immunization schedule, and to determine the number of extra immunization dose received by each child. Data was collected retrospectively from 528 children immunization cards in Iraq to obtain the immunization history of each individual child. This study was restricted the analyses to the vaccines administered before age 2 years. Each child must received seven doses at seven times, every dose consist of many types of vaccines. About 5.3% if immunization doses of 528 children were considered as extra immunization doses. More than 15% of extra immunization doses were shown in the sixth vaccination dose (MMR) at 15 months of child life. The majority of children (68.2%) were immunized without any extra immunization dose out of seven immunization doses. One hundred and forty-one children (26.7%) were immunized with one extra dose, while 27 children (5.1%) were immunized with two extra doses. This study found that compliance with WHO or national immunization recommendations is low and inappropriate immunization doses were occurring frequently. Any extra immunization dose will lead to increase in vaccine's adverse effect and increase in the vaccine's risk/benefit ration. It is very important to implement strategies that will lead to improved and developed immunization practice and childhood immunization coverage in the future.

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Evaluation of invalid vaccination

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Immunization doses were considered *invalid immunization doses* if they were administered before the minimal interval between-doses and had to be repeated. The aims of this study are to determine the frequency and percent of invalid doses among child immunization schedule, and to determine the number of invalid vaccination dose received by each child. Data was collected retrospectively from 528 children immunization cards in Iraq to obtain the immunization history of each individual child. This study was restricted the analyses to the vaccines administered before age 2 years. Each child must received seven doses at seven times, every dose consist of many types of vaccines. 8.3% of immunization doses of 528 children were considered as invalid immunization doses. More than 21% of invalid immunization doses were shown in the seventh or last vaccination dose (Measles) at 18 months of child life. The majority of children (54%) were immunized without any invalid immunization dose. One child only (0.2%) was immunized with six invalid doses, in addition, one child vaccinated with five invalid doses out of seven immunization doses. Each invalid immunization dose must repeat to increase vaccine benefit and decrease the risk of infectious disease. It is very important to implement strategies that will lead to improved and developed immunization practice and childhood immunization coverage in the future.

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Autologous vaccine *AHICE*®, cancer immunotherapy, colon-, mamma-, peritoneal-, pancreas-, small-cell-lung-ca., treatment results

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AHICE immunotherapy is distinguished by its unique selectivity and specificity against recognized tumors. The peculiarity of AHICE is the *demasking* of the tumor cells biochemically prior to vaccine preparation. Following that the autologous immune system detects and eliminates them spontaneously.

AHICE is being either sub cutan or i.v. administered.

Before and after AHICE start were examined: a differential blood count, a lymphocytes immune-phenotyping, the related tumor markers, TNF-α-, IFN-γ-concentrations. At the end of AHICE treatment the tumor situation was examined (MRI, CT or/and PET).

One colon-ca. overcomes the 6 years, is still living without neoplasies.

One pancreas ca. after surgery have had a rest life prolongation of over 9 years.

One peritoneal ca. have had remission (CT) in June 2004.

One breast ca. overcomes the 5 years living without neoplasies at best quality of life.

A small-cell lung-ca. (two brain metas, condition after radiation treatment, surgery of the lung tumors). No neoplasies were noticed in the lung, liver and the one brain-meta was melted down (over 2 years AHICE treatment-observation). The second brain-meta showed only a small peripheral region agent incorporating area (CT, MRI, PET). Excision of the tumor was carried out. Immunohistochemically showed multiple necrotic cells and increased CD56+ on cells (NKC's), as this is the proof of the *in vivo* effectiveness of AHICE.

In conclusion we can refer that after a previous demasking of tumor-cells, the so activated autologous immune system is the significant point of reference for successful cancer therapy.

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Immunoglobulin replacement in paediatric cases with secondary immunodeficiency following massive chylothorax- Leicester experience

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Introduction: Chylothorax poses a significant cause of morbidity in post congenital cardiac surgery (1). The incidence of chylothorax is likely to increase with complex repairs. Massive chylothorax following loss of immunoglobulin in chyle can lead to secondary immunodeficiency. The evidence available on management of secondary immunodeficiency following massive chylothorax is scarce (2).

Objectives: To look at patients with chylothorax, severity of chest drain losses, indications forimmunoglobulin supplementation, associated mortality and morbidity.

Methods: Retrospective case note review of patients with chylothorax from August 2009 to July 2011 in Paediatric Cardiac Intensive Care Unit.

Results: Total Number of patients with chylothorax = 9. Age ranged from 4 days old to 9 years. (<1 month = 5 patients, 1−12 month = 3 patients). Underlying cause of chylothorax: post cardiac surgery = 8, enterovirus myocarditis = 1. Number of patients where immunoglobulin were supplemented = 6. Number of patients with chest drain loss >25 ml/kg/day = 8. Number of patients with chest drain loss >80 ml/kg/day = 4. Highest chest drain loss 238 ml/kg/day in one patient. All supplemented patients had low IgG level, low lymphocyte count with low CD3, CD4, CD8 CD19 and B Cells level. Mortality = 2/6 in supplemented group. Higher chest drains losses associated with longer hospital stay day.

Summary: Secondary immunodeficiency following massive chylothorax further complicates the management of these complicated post cardiac surgery patients. This study highlights the importance of monitoring of immunoglobulin levels in patients with massive chylothorax.

Conclusion: Secondary immunodeficiency following massive chylothorax could increase morbidity and mortality; immunoglobulin replacement potentially helps to tie them through vulnerable period till chylothorax resolves pending normalisation of immune function.